MYCOLOGIA

OFFICIAL ORGAN OF THE MYCOLOGICAL SOCIETY OF AMERICA

Vol. XLIX JULY - AUGUST, 1957 No. 4

CONTENTS

[MYCOLOGIA for May–June (49: 305–447) was issued June 28, 1957.]

PUBLISHED BIMONTHLY FOR
THE NEW YORK BOTANICAL GARDEN
AT PRINCE AND LEMON STS., LANCASTER, PA.

Entered as second-class matter April 30, 1925, at the post office at Lancaster, Pa., under the Act of August 24, 1912.

MYCOLOGIA

Published by

THE NEW YORK BOTANICAL GARDEN

IN COLLABORATION WITH THE

MYCOLOGICAL SOCIETY OF AMERICA

MYCOLOGIA

OFFICIAL ORGAN OF THE MYCOLOGICAL SOCIETY OF AMERICA

VOL. XLIX JULY–AUGUST, 1957 NO. 4

MICROBIOLOGICAL PRODUCTION OF CAROTENOIDS. I. ZYGOSPORES AND CAROTENE PRODUCED BY INTRASPECIFIC AND INTERSPECIFIC CROSSES OF CHOANEPHORACEAE IN LIQUID MEDIA

C. W. HESSELTINE AND R. F. ANDERSON

Barnett, Lilly and Krause (1) reported that plus and minus strains of *Choanephora cucurbitarum* were capable of forming zygospores when grown together in liquid medium in shaken flasks. At the same time yields of 960 μg/g of β-carotene in dry mycelium were found. Numerous mating types of members of the Choanephoraceae were available in our Culture Collection including *C. cucurbitarum, C. trispora* (*Blakeslea trispora*), *C. conjuncta* and *Blakeslea circinans;* therefore, a study was made in which opposite mating types were grown singly and paired in liquid synthetic mucor medium (SMM) in shaken flasks. This medium is composed of glucose 40 g, asparagine 2 g, KH_2PO_4 0.5 g, $MgSO_4$ 0.25 g, thiamine 0.5 mg and 1 liter of distilled water. Five hundred-ml flasks containing 100 ml of this sterile medium or 100 ml of the medium used for sporulation of this family were inoculated with *C. cucurbitarum* strains NRRL A-6097 and NRRL A-6098 singly and paired. Portions of vegetative mycelium were used as inoculum. The flasks were placed on a rotary shaker and incubated for 72 hours at 28° C. At the end of the fermentation each strain grown singly resulted in masses of almost white-colored mycelium. However, in the flasks inoculated with + and − strains, the sporulation medium showed dark areas of zygospores while the vegetative mycelium was cream to

[MYCOLOGIA for May–June (**49**: 305–447) was issued June 28, 1957.]

pale yellow in color. In contrast the paired strains in SMM produced no zygospores but the mycelium was rolled into single large masses which were bright yellow-orange. Since these results looked interesting, further matings of available species of Choanephoraceae were made using potato dextrose (PD) and SMM.

The temperature of incubation was 28° C and the fermentation time was five days. Each fermentation was run in triplicate and the amount of inoculum was approximately equal whether single or paired strains were used. The method used in determining carotene was essentially the same as that used by Barnett *et al.* (**1**). Spectrophotometric measurement of the color at 450 mμ in the petroleum ether extract before

TABLE I

RESULTS OF MATING VARIOUS SPECIES OF THE CHOANEPHORACEAE IN SHAKEN FLASKS

Species	Strain number and mating reaction	Medium	Zygospores	Dry weight gm/300 ml	β-carotene	
					total μgm	μgm/gm
Choanephora cucurbitarum	A-6097 (+)×A-6098 (−)	SMM	−	1.29	200	155
	A-6097 (+)	SMM	−	1.09	60	55
	A-6098 (−)	SMM	−	1.13	52	50
	A-6097 (+)×A-6098 (−)	PD	+	1.25	395	320
C. trispora	NRRL 2456 (+)×NRRL 2457 (−)	SMM	−	0.67	368	550
	NRRL 2456 (+)	SMM	−	0.73	160	220
	NRRL 2457 (−)	SMM	−	0.58	90	155
	NRRL 2456 (+)×NRRL 2457 (−)	PD	+	1.21	700	580
C. conjuncta	NRRL 2560 (+)×NRRL 2561 (−)	SMM	−	0.72	133	185
	NRRL 2560 (+)	SMM	−	0.71	18	25
	NRRL 2561 (−)	SMM	−	0.86	146	170
	NRRL 2560 (+)×NRRL 2561 (−)	PD	+	0.99	143	145
Blakeslea circinans	NRRL 2546 (+)×NRRL 2548 (−)	SMM	−	0.75	255	340
	NRRL 2546 (+)	SMM	−	0.82	20	25
	NRRL 2548 (−)	SMM	−	0.69	35	50
	NRRL 2546 (+)×NRRL 2548 (−)	PD	+	1.15	286	250

and after saponification showed less than 20% loss in color in the saponification step. Chromatographic separations, according to the A.O.A.C. method (**2**), indicated that the crude pigments produced by the mated cultures contained about 80% β-carotene plus smaller amounts of other carotenes. The results are shown in TABLE I. The results prove that all species of the Choanephoraceae grown upon PD liquid medium produced zygospores but none was formed on the synthetic medium. All four species available for study produced β-carotene. When paired mating types were grown on the SMM, three of the four species produced at least twice as much β-carotene as either parent strain grown by itself and even in the fourth species on a weight basis, the paired strains yielded β-carotene in excess of either parent.

It had been observed (3) that when opposite mating types of two different species were mated upon potato dextrose agar, those of the same species produced many zygospores and the yellow pigment seen in the early stages of zygospore formation disappeared. However, when one strain of one mating type was mated with an opposite mating type belonging to another species, large amounts of yellow pigment appeared at the point of union of the two mated strains and this color persisted. We thought it might be interesting to make these interspecific matings under the same conditions as those in TABLE I. The results of three cross matings are shown in TABLE II. Again it will be noted that the yields of product from the mated strains were far greater than those seen in either of the mating types as seen in TABLE I. In the first two

TABLE II

RESULTS OF MATING STRAINS OF CHOANEPHORA OF OPPOSITE MATING TYPES FROM DIFFERENT SPECIES IN SHAKEN FLASKS USING SMM MEDIUM

Species	Strain and mating reaction	Dry weight gm/300 ml	β-carotene	
			total μgm	μgm/gm
C. cucurbitarum and *C. trispora*	A-6097 (+) × NRRL 2457 (−)	1.16	215	185
C. trispora and *C. conjuncta*	NRRL 2457 (−) × NRRL 2560 (+)	0.73	212	290
C. conjuncta and *C. cucurbitarum*	NRRL 2562 (−) × A-6097 (+)	0.95	265	280

of these interspecific crosses, the yield of β-carotene is intermediate in amount when compared with those of the intraspecific parent matings as seen in TABLE I.

Thus we have shown that all available species of the Choanephoraceae produce zygospores when opposite mating types are inoculated together in appropriate liquid media. Yields of β-carotene are much higher in the mated strains than in either parent grown alone. Not only is this true within a species but it also occurs when opposite mating types of different species are mated.

NORTHERN REGIONAL RESEARCH LABORATORY
AGRICULTURAL RESEARCH SERVICE
UNITED STATES DEPARTMENT OF AGRICULTURE
PEORIA, ILLINOIS

REFERENCES

1. **Barnett, H. L., V. G. Lilly and R. F. Krause.** Increased production of carotene by mixed + and − cultures of *Choanephora cucurbitarum*. Science **123**: 141. 1956.
2. Methods of Analysis—Association of Official Agricultural Chemists: Sixth Edition, Washington, D. C., 1945.
3. **Hesseltine, C. W. and C. R. Benjamin.** Notes on the Choanephoraceae. Mycologia **49**. In press.

STUDIES ON THE SYNTHESIS OF ALPHA-AMYLASE AND FREE AMINO ACIDS BY MUTANTS OF ASPERGILLUS ORYZAE [1]

HO SIK KIM, W. F. GEDDES, AND J. E. DEVAY [2]

Increased production of certain organic acids, enzymes, and antibiotics by fungi which can synthesize these substances has been accomplished in many instances by inducing mutations in the parent strains. A family of selected strains of *Penicillium chrysogenum* has been produced by Backus and Stauffer (**3**) who used ultra-violet radiation, X-rays, nitrogen mustard, and other chemicals to produce mutants which can synthesize greater amounts of penicillin than the original strains. Diller *et al.* (**6**) produced a mutant of *Aspergillus niger,* using X-rays, which fermented glucose to citric acid more efficiently than the parent. Raper *et al.* (**10**) found that the production of itaconic acid by a mutant of *Aspergillus terreus,* induced with ultra-violet radiation, was greater than that produced by the parent. In other studies, Nobuyoshi (**8**) isolated 2 mutants of *Aspergillus oryzae,* induced with ultra-violet radiation, which surpassed the original strain in amylase activity by 100%, and in protease activity by 50%. Maxwell (**7**), using ultra-violet radiation, also induced a mutant in *A. oryzae* which produced more protease than the parent strain. Supersonic sound waves were used by Oda *et al.* (**9**) to produce variants in *A. oryzae* which had greater amylase and protease activity than the parent strains.

In this investigation, the amylase-producing potentials of industrially important strains of *Aspergillus* spp. were studied. Additional studies were made on the amino-acid metabolism of mutants of *A. oryzae.*

MATERIALS AND METHODS

Isolation of Aspergillus spp. The following monoconidial isolates of *Aspergillus* spp. were obtained from 6 different sources of moldy rice

[1] Paper No. 3618, Scientific Journal Series, Minnesota Agricultural Experiment Station.

[2] Professor and Head, Department of Agricultural Chemistry, Seoul National University, Suwon, Korea (formerly on leave of absence at University of Minnesota); Professor and Head, Department of Agricultural Biochemistry, University of Minnesota; and Assistant Professor, Department of Plant Pathology and Botany, University of Minnesota, respectively.

brans and 5 different samples of moldy rice: *A. oryzae* (Ahlburg) Cohn, 14 isolates; *A. awamori* Nakazawa, 3 isolates; and *A. wentii* Wehmer, 7 isolates.

Production of mutants with ultra-violet radiation. Spore suspensions were prepared by adding 5 ml of sterile water to sporulating colonies of *Aspergillus* spp. on potato-dextrose agar and then shaking the cultures to dislodge the spores. These suspensions were transferred to sterile bottles and diluted to 15 ml with sterile water. The spore suspensions were then poured into sterile Petri dishes and agitated with a magnetic stirrer during irradiation. An ultra-violet lamp (2537Å, Westinghouse type SB Sterilamp) was mounted 12 mm above the spore suspensions. Periods of irradiation were 5, 10, 15, 20, 25, and/or 30 minutes. The temperature of the cell suspensions during irradiation varied from 25° to 30° C. After irradiation, 1 ml of a treated spore suspension was pipetted into a dilution bottle which contained 99 ml of sterile water. Aliquots of 1 ml of diluted spore suspensions were then pipetted onto 20 ml amounts of the following medium, designated NM-1, in Petri dishes: NH_4NO_3, 0.5 gm; K_2HPO_4, 1 gm; $MgSO_4 \cdot 7H_2O$, 0.5 gm; soluble starch, 2 gm; agar, 20 gm; and H_2O 1 liter.

After incubation for 3 to 4 days at room temperature (25° C), 1 ml of an approximately 0.1 N iodine solution was carefully distributed around each of the 10 to 20 colonies per plate. After approximately 10 minutes, colonies surrounded by achromatic zones wider than those around colonies of the parent isolates were chosen for further study.

Reselections of these colonies were made by planting a biscuit, 5 mm in diameter, of a selected colony in the center of a Petri dish containing 20 ml of medium NM-1. After 5 days, the iodine test for starch was made and the size of the achromatic zone surrounding the resulting colony was measured.

Production of mutants with polonium^{210}. Conidiospores of the various isolates were suspended in water as previously described. A loopful of spores from a suitable dilution was then deposited on the center of a Petri dish containing 20 ml of the starch medium NM-1. An unglazed cover was placed over the dish to dry the agar surface around the spores after which they were exposed to a plate 8–10 mm above the spores having 4.8 millicuries of polonium^{210} activity. For dry spores the lethal dosage exceeded 70 minutes; however, when the spores were moistened by keeping them in a water-saturated chamber overnight, they were more sensitive to the radiation. Exposures of 10, 20, 30, and/or 40 minutes were made and it was found that at 40 minutes approximately 90–95% of the spores were killed.

After irradiation, 1 ml of sterile water was pipetted on the plate and the treated spores were evenly distributed on the agar surface. An unglazed cover was placed over the plate to dry the agar surface and the plates were incubated at room temperature (approximately 25° C). The method of selecting mutants was the same as previously described.

Further and more accurate quantitative tests for α-amylase activity of selected mutants were made using liquefying and dextrinizing methods.

Starch liquefying tests. The starch liquefying activity of the various preparations was measured by means of the Brabender Amylograph, a recording torsion viscometer which provides a continuous automatic record of the changes in the viscosity of a starch suspension as the temperature is increased at a uniform rate of about 1.5° C per minute. The increase in viscosity which takes place upon gelatinization of the starch is opposed by the liquefying action of the amylase, and the height of the curves at maximum viscosity is an index of the liquefying activity. Amylograms were made employing 50 gm of wheat starch suspended in 450 ml 0.1 M acetate buffer (pH 4.8) as described by Anker and Geddes (**2**). The temperature of the starch suspension was allowed to increase to 95° C where it was held constant. The apparatus was usually operated for a total elapsed time of 45 minutes.

Standard amylograms were made by adding different amounts of an amylase preparation, Rhozyme A-4 (Rohm and Haas Co., Philadelphia), which had an α-amylase activity of 5000 Sandstedt Kneen-Blish units per gram. (An S.K.B. unit (**11**) is the number of grams of soluble starch which, under the influence of an excess of β-amylase, are dextrinized by 1 gm of enzyme source in 1 hour at 30° C.) The peaks of the curves corresponding to various amounts of the standard amylase preparation were as follows:

Rhozyme A-4 added gm	Amylogram peaks Brabender units
1.05	208
0.225	735
0.150	795
0.100	848
0.075	870
0	975

In order to determine the amount of α-amylase produced by various monoconidial isolates, duplicate cultures were grown in 500 ml Erlenmeyer flasks with 250 ml of a medium, designated NM-2, containing $Ca(NO_3)_2 \cdot 4H_2O$, 0.333 gm; $MgSO_4 \cdot 7H_2O$, 0.083 gm; KNO_3, 0.083 gm; KH_2PO_4, 0.083 gm; KCl, 0.042 gm; $FeCl_3 \cdot 6H_2O$, 0.24 mg; $ZnCl_2$, 0.15 mg; H_3BO_3, 0.06 mg; $CuCl_2 \cdot 2H_2O$, 0.05 mg; $MnCl_2 \cdot 4H_2O$, 0.04

mg; $NaMoO_4 \cdot 2H_2O$, 0.03 mg; dextrose, 10 gm; soluble starch, 0.1 gm; and distilled water to 1 liter. Flasks were capped with filter paper and the medium autoclaved for 20 minutes at 121° C. Mycelial biscuits were planted in the nutrient solution and the cultures incubated at 25° C on a rotary action shaker for 8 days and then kept stationary for 6 more days.

Amylase activity of the cultures was determined by adjusting 200 ml of a culture filtrate to pH 4.8 and buffering the solution with 250 ml of an acetate buffer (0.1 M) of the same pH to make a total volume of 450 ml. The starch suspension was then prepared and tested in the amylograph using this buffered culture filtrate. The concentration of amylase in the cultures was then estimated using values from the standard amylograms for different concentrations of Rhozyme A-4.

α-amylase activity. α-amylase activity was determined in culture filtrates and enzyme extracts of moldy rice using the dextrinizing procedure of Sandstedt *et al.* (**11**) as described in Cereal Laboratory Methods (**1**) with the exception that the absorbance of the standard dextrin-iodine solution at 650 mμ was used as the end point of the reaction. Walden (**12**) has shown that at this wave length the absorbance of the dextrin-iodine complex determined at various digestion times of the buffered limit dextrin substrate with α-amylase is proportional to the digestion time. From the absorbance of the standard-dextrin iodine solution and that of the digestion mixture at a given time, the α-amylase activity could be readily calculated.

A sample of Rhozyme A-4 (Rohm and Haas), with an α-amylase activity of 5000 S.K.B. units per gram when purchased, was analyzed to check the accuracy of the method. At the time of analysis, the activity of the sample was determined to be 4700 S.K.B. units per gram.

Culture methods. Liquid cultures of the parent and mutant isolates were prepared in the same manner as those used for the amylograph tests except that 2 kinds of media were used and the cultures were stationary. Duplicate cultures of each isolate grown in the medium NM-2 were incubated at 25° C for 11 days. Cultures grown in Corman's medium (**4**), designated NM-3, which contained NaCl, 3.00 gm; $MgSO_4 \cdot 7H_2O$, 3.00 gm; $NaNO_2$, 4.44 gm; NH_4NO_3, 1.71 gm; K_2HPO_4, 26.1 gm; KH_2PO_4, 20.4 gm; $FeSO_4 \cdot 4H_2O$, 0.6 mg; $ZnSO_4 \cdot 7H_2O$, 0.6 mg; $MnSO_4 \cdot 4H_2O$, 0.6 mg; soluble starch, 45.0 gm; and distilled water to 3 liters, were incubated for 8 and/or 12 days at 25° C. Various amounts up to 10 ml of the culture filtrates were added to 20 ml of the β-limit dextrine substrate and the reaction mixture made up to a final volume of 30 ml with water and incubated as previously

described. After varying intervals, 1 ml aliquots were withdrawn and analyzed for α-amylase activity.

When grown on a grain medium, 1 ml of mycelium and spores of selected cultures were pipetted onto 100 gm (dry weight) of autoclaved rice which had been previously steeped for 2 hours in water. Duplicate cultures were incubated for 5 days at 25° C. The moldy rice was then air-dried and ground in a Wiley mill to pass a No. 60 mesh screen. One gram of the pulverized rice was then extracted overnight at 25° C with 10 ml of 0.2% NaCl solution and 0.1 ml of toluene. The extract was filtered and tested for α-amylase activity.

Amino acid analyses. The nature of free amino acids in culture extracts was ascertained to investigate possible relationships between the metabolism of free amino acids and amylase activity. Ten subcultures of each isolate were grown in 250 ml Erlenmeyer flasks containing 50 ml of the nutrient medium, NM-2. The cultures were kept sta-

TABLE I

WIDTH OF ACHROMATIC ZONES AROUND COLONIES OF SELECTED MUTANTS OF ASPERGILLUS ORYZAE, ISOLATE 6A

Isolate	Achromatic zone	Colony diameter
6A20M8[a]	18 mm[b]	21 mm[c]
6A20M1	11 mm	19 mm
6A	8 mm	19 mm

[a] Mutants are designated first by isolate number of parent, then the number of minutes of exposure to polonium radiation, and finally by a colony number.

[b,c] Average value from 5 different plates in which colonies started from 5 mm biscuits of mycelium and spores.

tionary and incubated for 12 days at 25° C; those of each isolate were then combined and diluted with 95% ethyl alcohol to give a final alcohol concentration of 70%. The combined cultures of the different isolates were then agitated in a Waring blendor for 3 minutes and extracted at 3° C for 24 hours. The extracts were clarified by centrifugation and then concentrated to near dryness at 50° C under partial vacuum. The residue of each extract was dissolved in 10 ml of 70% ethyl alcohol and the resulting concentrate of free amino acids analyzed by descending paper partition chromatography according to the method of Dent (**5**). A 2-dimensional chromatogram was made of each extract employing a total of 150 μl using phenol:water (1:1) as the first solvent and a mixture of 2,4-lutidine:2,4,6-collidine:water (1:1:2) as the second solvent. After irrigation at 25° C for 24 and 40 hours, respectively, in each dimension, and drying, the chromatograms were developed by spraying with a ninhydrin solution (0.4 gm triketo-hydrindene hydrate and 10 gm

phenol per 100 ml 70% aqueous ethanol). The color intensity of the resulting spots was increased by heating the chromatograms in an oven at 85° C for 5 minutes. Tentative identifications of the spots were made by comparing the R_f values of the amino acids in a known mixture with those in the culture extracts, by characteristic spot colors, and by superimposing known mixtures of amino acids on the spots of the culture extracts before irrigating the chromatograms.

RESULTS

Production of mutants. Irradiation of the 24 original isolates of *Aspergillus* spp. with ultra-violet rays and polonium^{210} resulted in the production of mutants which differed from the parent isolates in amylase synthesis, growth, sporulation, colony pigmentation, and in the accumulation of different amounts of free amino acids in liquid cultures.

TABLE II

ESTIMATIONS OF α–AMYLASE ACTIVITY IN CULTURE FILTRATES OF MUTANTS OF ASPERGILLUS ORYZAE, ISOLATE 6A, WITH THE AMYLOGRAPH METHOD

Culture[a]	Final culture pH	Peaks of amylograms	Equivalent milligrams of Rhozyme A-4 estimated per ml of culture filtrate[b]
6A20M8	4.8	760	0.980
6A20M1	4.8	830	0.595
6A	4.2	860	0.430

[a] Duplicate cultures were grown in 250 ml amounts of medium NM-2 at 25° C on a rotary action shaker for 8 days, then kept stationary 6 more days.

[b] An average value of 0.0011 gm of Rhozyme A-4 per change in 1 amylograph unit between 735 and 870 was used for these estimations.

Of all the isolates irradiated, 6A of *A. oryzae* was the most mutable. As a result of treatment of this fungus with ultra-violet radiation followed by screening tests for mutations in amylase production and other characteristics, 2 mutants were obtained whose growth on semi-solid as well as in liquid media was much less than that of the parent, 6A. No mutations for amylase synthesis were obtained from isolates treated with ultra-violet radiation.

Irradiation of isolate 6A with polonium^{210} resulted in the selection and isolation of at least 25 mutants for increased amylase production from which two, 6A20M1 and 6A20M8, were reselected for further studies (TABLE I). The basis for designated various isolates as mutants was the persistence of their acquired characteristics through successive transfer generations on laboratory media. No reversions to the parent type were found in any of the mutants.

TABLE III

ESTIMATIONS OF α-AMYLASE ACTIVITY IN CULTURE FILTRATES OF MUTANTS OF ASPERGILLUS ORYZAE, ISOLATE 6A, WITH DEXTRINIZATION TESTS

Isolate	NM-3[a]				Steeped rice[b]
	Culture age: 8 days		Culture age: 12 days		
	Final pH	α-amylase SKB units/ml filtrate	Final pH	α-amylase SKB units/ml filtrate	α-amylase SKB units/gm moldy rice
6A20M8	6.6	1.00	6.4	5.2	350
6A20M1	6.7	0.46	6.4	4.0	250
6A	6.2	0.35	6.2	2.6	190

[a,b] Cultures of each isolate were grown at 25° C and kept stationary. Each figure is an average of tests on duplicate cultures.

Liquefying activity. The α-amylase production by isolates 6A, 6A20M8, and 6A20M1 of *A. oryzae* as measured by the amylograph technique is summarized in TABLE II, together with the final pH of the cultures. These results indicate that culture filtrates of mutant 6A20M8 contained approximately 2.3 times as much α-amylase as the parent 6A.

Dextrinization tests. Isolates 6A, 6A20M8, and 6A20M1 were compared for α-amylase synthesis by growing them in nutrient solutions NM-2 and NM-3 and on a rice substrate. In these tests the liquid

TABLE IV

FREE AMINO ACIDS IN CULTURE FILTRATES OF MUTANTS OF ASPERGILLUS ORYZAE, ISOLATE 6A

Amino acids	Isolates of *A. oryzae*			
	6A	6A20M8	6A5M22	6A10M32
glutamic acid	+[a]	+	+	+
alanine	+	+	+	+
valine	+	+	+	+
leucine and/or isoleucine	+	+	+	+
γ-NH_2 butyric acid	+	+	+	+
glutamine	−	+	+	−
proline	−	+	−	−
serine	−	−	+	−
glycine	−	+	−	−
tyrosine	−	+	−	−
lysine	−	+	+	+
arginine	−	+	+	+

[a] Plus signs indicate the the amino acids were identified on chromatograms of culture filtrates. Minus signs indicate that the amino acids were not present in sufficient concentrations to be recognized by the methods used.

cultures were kept stationary. At the end of the growth period, the pH and α-amylase activity of the cultures were determined. The reactions of cultures grown in NM-2 were between pH 3.2 and 3.5 and subsequent dextrinizing tests showed negligible amounts of α-amylase activity in filtrates of these cultures. In contrast, filtrates of cultures grown in NM-3 for 8 or 12 days had higher pH values and greater α-amylase activities (TABLE III). Comparisons are also given in TABLE III of the α-amylase activity in cultures of these isolates when grown in a rice medium.

It is evident from these results that cultures of mutant 6A20M8 had approximately 2 times the α-amylase activity of the cultures of its parent, 6A. As in the other tests, cultures of mutant 6A20M1 were intermediate between 6A20M8 and 6A in α-amylase activity.

Amino acid analyses. Associated with mutations for amylase synthesis and growth in isolate 6A of *A. oryzae* were changes in amino acid metabolism. The results of chromatographic analyses for free amino acids in culture filtrates of slow-growing mutants, 6A10M32 and 6A5M22, as well as 6A20M8, which produced greater amounts of α-amylase than 6A, are given in TABLE IV.

DISCUSSION

Associated with increased α-amylase activity in cultures of mutant 6A20M8 were greater concentrations (visual estimations from 2-dimensional chromatograms) of the basic amino acids, arginine and lysine, as well as glutamine, glycine, proline, and tyrosine. The relationship of different kinds and amounts of free amino acids in the cultures and α-amylase activity was not apparent; however, the concentrations of various amino acids may have directly or indirectly influenced the culture pH. Cultures of *A. oryzae* whose pH was 3.2 to 3.4 had little or no amylase activity whereas cultures growing on the same medium but harvested when the culture pH was 4.8 had much greater amylase activity. However, cultures having a pH of 6.2 at the end of 8 days had much less amylase activity than cultures growing on the same medium with the same pH at the end of 12 days. α-amylase is destroyed at approximately pH 3, which may account for its low activity in certain cultures.

It was evident that mutants of *A. oryzae,* even though greatly affected by culture conditions, always placed in the same relative order when compared for amylase production, indicating the constancy of these mutations.

SUMMARY

The effectiveness of ultra-violet rays and α radiation from polonium^{210} in causing mutations affecting the synthesis of α-amylase in 24 industrially important monoconidial isolates of *Aspergillus* spp. was studied. Mutants were obtained from *Aspergillus oryzae* (Ahlburg) Cohn, isolate 6A, whose cultures differed from 6A in growth, sporulation and α-amylase activity. α-amylase production by mutants 6A20M1 and 6A20M8 was quantitatively determined by amylograph and dextrinization methods. Isolate 6A20M8 produced more than twice as much α-amylase as the parent isolate 6A. Chromatographic analyses of culture filtrates of the various mutants and parent isolates indicated that mutations which affected amylase synthesis also influenced the metabolism of free amino acids by these fungi.

Grateful acknowledgments are made to Robert J. Klug, George C. Papavizas, and John F. Tuite of the Department of Plant Pathology and Botany, and to Neville Prentice of the Department of Agricultural Biochemistry for their assistance in certain phases of this research.

DEPARTMENT OF AGRICULTURAL BIOCHEMISTRY
AND
DEPARTMENT OF PLANT PATHOLOGY AND BOTANY
INSTITUTE OF AGRICULTURE
UNIVERSITY OF MINNESOTA
ST. PAUL 1, MINNESOTA

LITERATURE CITED

1. American Association of Cereal Chemists. 1947. Cereal Laboratory Methods. Ed. 5. The Association: St. Paul, Minnesota.
2. **Anker, C. A. and W. F. Geddes.** 1944. Gelatinization studies upon wheat and other starches with the amylograph. Cereal Chem. **21**: 335–360.
3. **Backus, M. P. and J. F. Stauffer.** 1955. The production and selection of a family of strains in *Penicillium chrysogenum*. Mycologia **47**: 429–463.
4. **Corman, J.** 1951. Enzyme production. United States Patent Nos. 2, 557, 078.
5. **Dent, C. E.** 1948. A study of the behavior of some sixty amino-acids and other ninhydrin-reacting substances on phenol "collidine" filter paper chromatograms, with notes as to the occurrence of some of them in biological fluids. Biochem. Jour. **43**: 169–180.
6. **Diller, Violet M., Alice G. Tytell, A. Tytell and H. Kersten.** 1950. A soft x-ray mutant of *Aspergillus niger*. Plant Physiol. **25**: 340–353.
7. **Maxwell, M. E.** 1952. Enzymes of *Aspergillus oryzae* II. The yield of enzymes from mutants produced by ultraviolet irradiation. Australian Jour. Sci. Res. B **5**: 56–63.
8. **Nobuyoshi, I.** 1951. Studies on Aspergillus III. Jour. Agr. Chem. Soc. Japan **24**: 283–287. Biol. Abs. **26**: 35385.

9. **Oda, M., S. Kamo, H. Takiguchi, A. Nunotani and K. Yamagata.** 1952. Variation of *Aspergillus oryzae* I. The induced variation 1. Jour. Fermentation Technol. (Japan) **30**: 120–128. Chem. Abs. **46**: 3450.
10. **Raper, K. B., R. D. Coghill and A. Hollaender.** 1945. The production and characterization of ultraviolet induced mutations in *Aspergillus terreus* II. Cultural and morphological characteristics of the mutations. American Jour. Bot. **32**: 226–236.
11. **Sandstedt, R. M., E. Kneen and M. J. Blish.** 1939. A standardized Wohlgemuth procedure for alpha-amylase activity. Cereal Chem. **16**: 713–723.
12. **Walden, C. C.** 1954. Purification of wheat alpha- and beta-amylase. Ph.D. Thesis, University of Minnesota. 160 p.

ENTEROBRYUS ATTENUATUS FROM THE PASSALID BEETLE[1]

ROBERT W. LICHTWARDT

(WITH 19 FIGURES)

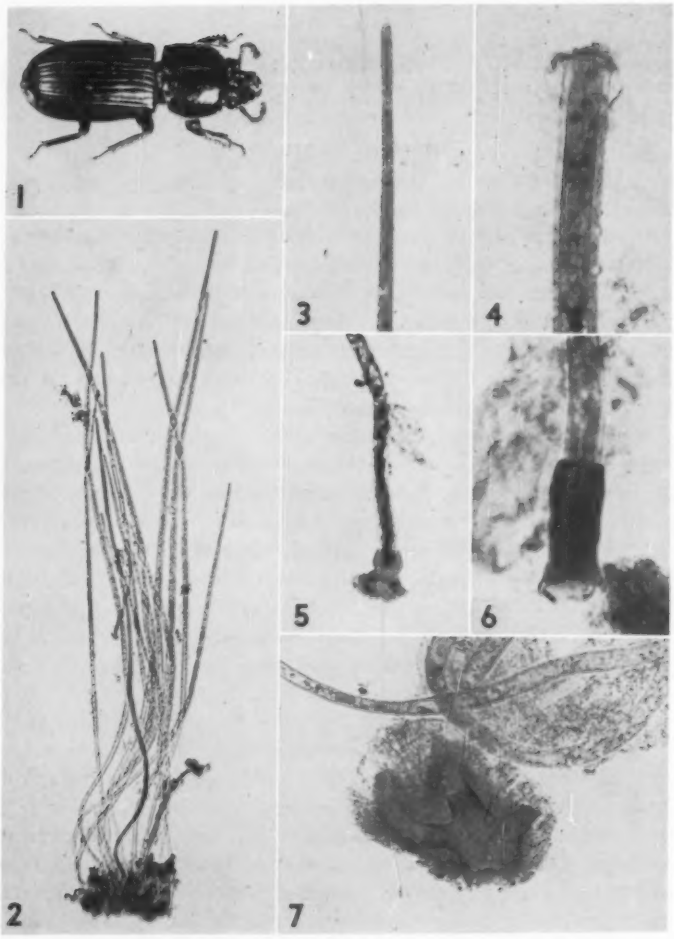
Enterobryus is one of the genera of commensal fungi belonging to the order Eccrinales, and, like all members of the order, its species have been found living only within the hindguts of arthropods. While the sexual nature of species within the Eccrinales has not been clearly established, their vegetative characteristics suggest possible affinities with the Phycomycetes, for the hyphae are coenocytic, non-septate, and the presence of cellulose can be demonstrated in the cell walls.

Enterobryus attenuatus, one of five species described by Joseph Leidy between 1849 and 1853, is of particular interest because it was discovered in the hindgut of a beetle, *Passalus cornutus* Fab., rather than in millipeds as were the other species found by Leidy. This host difference has led some authors to make a generic separation of those species found in beetles from those found in millipeds, a matter which will be discussed later in this paper. *E. attenuatus* was first described in a paper presented by Leidy in **1849(b)**, though mention of this species had been made in a paper published earlier that same year (**1849a**). A more complete description with drawings appeared in **1853**.

I have collected specimens of *Passalus cornutus* and have studied the fungi associated with them. Hyphae of the fungus which Leidy discovered were seen in all individuals, and were found to be substantially as Leidy described them. However, in the most anterior region of the hindgut of all host specimens dissected I also found other hyphae which, upon first examination, appeared to be quite different from those described by Leidy, but it is my belief that they represent another phase of the fungus, *Enterobryus attenuatus,* apparently not observed by Leidy. It is the purpose of this paper to present a description of these new hyphal types and the spores they produce, and to suggest how they fit into the life cycle of the fungus.

[1] This paper is based upon a portion of a Ph.D. thesis submitted to the University of Illinois in 1954. The author is greatly indebted to Dr. Leland Shanor who supervised this research.

Journal Paper No. J-3127 of the Iowa Agricultural Experiment Station, Ames, Iowa. Project No. 1300.



FIGS. 1–7. Posterior hyphae of *Enterobryus attenuatus*, and beetle host. 1. *Passalus cornutus* Fab., host of *E. attenuatus*, × 1. 2. Tuft of hyphae from pocket in colon of hindgut, × 60. 3. Fusiform tip of mature vegetative hypha, × 460. 4. Blunt tip of thick-walled hypha which apparently has sporulated, × 460. 5. Holdfast of young hypha, × 460. 6. Single holdfast of mature hypha, × 460. 7. Fused holdfasts, × 200.

MATERIALS AND METHODS

The beetle *Passalus cornutus* (FIG. 1) is a relatively common and widespread species of insect in the United States. Specimens can be collected in or near rotting logs within which the adults form a crude network of tunnels where they may live gregariously with their larvae. Host material for the present study was collected in Johnson and Pope Counties, Illinois, and Madison and St. Francois Counties, Missouri.

Since species of *Enterobryus* have not yet been cultured successfully outside of their hosts, all observations were made directly from freshly dissected beetles. The hindgut of the beetle can be removed by cutting open the abdomen. A more rapid means of removing the hindgut, requiring a little more experience, consists of grasping the anal region of the beetle with a pair of forceps and carefully tearing out the entire hindgut. An advantage of this second method is that the beetle remains almost intact externally, which may be desirable if the host is to be preserved. The excised hindgut can be cut open and the fungus material studied in the living condition, or the lining of the gut with attached fungi can be removed according to the methods outlined in a previous paper (Lichtwardt, **1954**). For the present study all the material eventually was mounted on microscope slides in lactophenol containing cotton blue.

Adult beetles possess a much-coiled and twisted alimentary canal. The length of the hindgut measures 4–4.5 cm, which is 0.5 to 1 cm longer than the beetle itself. The hindgut is divided into three distinct regions. The foremost of these, the ileum, is 4 or 5 mm in length and is lined with the usual chitinous substance found in hindguts. The lining here has many small and some large thorny protrusions (FIG. 10) which may prevent detail in hyphae attached to the lining from being clearly visible. The mid-region of the hindgut, the colon, is more enlarged than the rest of the gut, and throughout its length, about 18 mm, it is regularly lobed with deep pockets. The gut lining in this region can be removed, but it is almost opaque when seen through the microscope, due to the large numbers of bacteria, the presence of debris, and the brownish color of the lining itself. The last and narrowest region of the hindgut is an elongated rectum of about 20 mm in length, and the lining from this part is essentially transparent.

In all the specimens of *Passalus cornutus* examined, hyphae of *Enterobryus attenuatus* have been observed attached to the lining of the ileum and colon, but not the rectum. To observe the hyphae from the first region, the lining of the ileum can be mounted on a slide in its entirety or in pieces with the attached fungi, or the hyphae can be picked

off with a very fine needle and mounted without too much difficulty. To study the hyphae from the colon it is necessary in almost all cases, because of the opaqueness of the lining, to mount detached hyphae or very small pieces of the lining to which they are attached.

OBSERVATIONS

The most pertinent part of Leidy's formal description of *Enterobryus attenuatus* in his paper of **1853** (p. 20) states:

"*Thallus* faintly brownish, yellowish, or hyaline; forming at first a double flexure or sigmoid curve, and then proceeding in a straight or gentle curvilinear direction to its free extremity. *Pedicle* short, cylindroid, campanulate, or conical with a spreading base, longitudinally striated, simple, occasionally double, uniformly yellowish. *Principal cell* cylindrical, attenuated at both extremities, or very slightly and gradually narrowing from the commencement, or uniform throughout; truncated, or obtusely rounded at the free extremity. *Terminal cells* rare."

This description rather accurately fits the hyphae observed by me in the colon of the Passalid beetle, and Leidy's drawings leave little doubt that the same fungi are being considered here. Apparently either Leidy did not observe the morphologically different and somewhat smaller hyphae attached in whorls to the ileum, described in the following pages, or the hosts which he examined were void of them.

THE POSTERIOR HYPHAE

The hyphae attached to the colon (FIGS. 2–7), which may be referred to as the posterior hyphae, show a definite tendency to aggregate. While individual hyphae are found, they are more readily seen as white tufts projecting from the inside surface of the very deeply and regularly lobed walls of the gut. As stated previously, conditions are such that they can be studied under higher magnifications only when removed from the gut lining. Even when removed, they are intimately surrounded by masses of bacteria and intestinal debris which must be cleared away. This material is especially abundant near the bases of the hyphae (FIGS. 2, 7). Individual filaments of bacteria may be found attached to hyphae, and in some instances hyphae are almost completely ensheathed in a substance which may be partly shed when the material is killed and fixed.

The holdfast is quite characteristic for these hyphae, the filament giving the appearance of being embedded in the holdfast (FIGS. 5, 6). Within a tuft of hyphae one occasionally can find some of the holdfasts fused (FIG. 7). Leidy states (**1853**) that in two instances he observed

the holdfast "divided at the summit," and he illustrates this phenomenon in one of his drawings. As will be pointed out, this fusion of holdfasts is of frequent occurrence among the anterior hyphae.

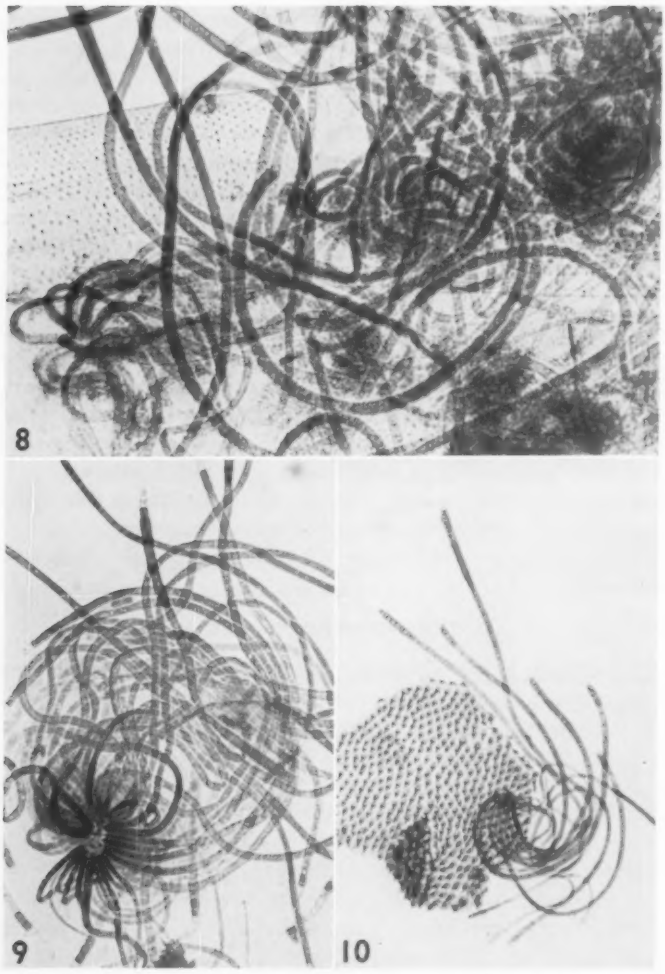
Starting from the area of attachment, a hypha rises in a mildly undulating fashion, then becomes essentially straight, or it may be moderately erect throughout its length. The filaments are fusiform in shape, though it is predominantly the unattached end that appears narrowed (FIG. 3). The nuclei in the posterior hyphae generally measure 3.5–4 μ. The walls of mature hyphae may be hyaline to brownish in color and have a thickness of 1–2 μ, though hyphae with much thicker walls may be present.

The absence of spores in these hyphae is of particular interest. I have not found spores, terminal cells, or other structures that might be indicative of reproduction, although some hyphae have blunt, rounded tips (FIG. 4) which must have resulted from the loss of the apical portion of the hypha. Leidy states (**1853**, p. 25) that in only six instances, among the several thousand filaments of *Enterobryus attenuatus* which he observed, was he able to detect the existence of a terminal cell.[2] It is apparent that either spore production by the hyphae in this region of the gut is rare, or that when spore production does occur it is completed rapidly or during a period in the life cycle of the host which has not been examined.

THE ANTERIOR HYPHAE

The anterior hyphae from the ileum may be quite inconspicuous if not abundant, although their presence usually can be detected with a good, high-powered dissecting microscope. These filaments may grow singly, but more often are found in clusters of a few to many dozens of hyphae, radiating in wide, graceful curves from the area of attachment (FIGS. 8–10). When in clusters, the holdfasts show the characteristic of being fused, as is clearly shown in FIGS. 11 and 12. Fusion of holdfasts to form a compound structure of this type has not been reported in any other species of Eccrinales. The compound holdfast is attached to the gut lining at one broad point, and if care is exercised the entire cluster of hyphae can be removed intact. Ordinarily there are large masses of debris and bacteria associated with the hyphae, especially near their bases, and removal of these accumulations is required in order to study the structure of the holdfast.

[2] In Leidy's drawings he illustrates two hyphal tips with crosswalls and states (**1853**, p. 59) that these are the only *two* instances of terminal cells (sporangia) he has observed.



FIGS. 8–10. Anterior hyphae of *Enterobryus attenuatus*. 8. Three whorled clusters of hyphae attached to chitinous lining of ileum, × 140. 9. Top view of clustered hyphae showing center of attachment, × 100. 10. Small cluster of hyphae attached to a spiny piece of lining of ileum, × 90.

Two types of hyphae appear to be present in clusters: wider hyphae measuring 8–16 μ in width, commonly around 12 μ, and narrower hyphae averaging about 7 μ wide but ranging from about 5 to 10 μ. The wider hyphae usually predominate in number, and clusters may be composed entirely of this type. The hyphal walls usually have a thickness of 0.5–1 μ, and are smooth and colorless. At the base of each hypha there is a dark, yellowish-brown disk (FIG. 12), measuring about 3.5–4.5 μ in diameter, from which the holdfast arises. When hyphae are pulled, the hyphae frequently break away from the holdfast just below this disk. In many other species of *Enterobryus*, which have no such disk, the holdfast would separate from the gut lining before it would break away from the hypha.

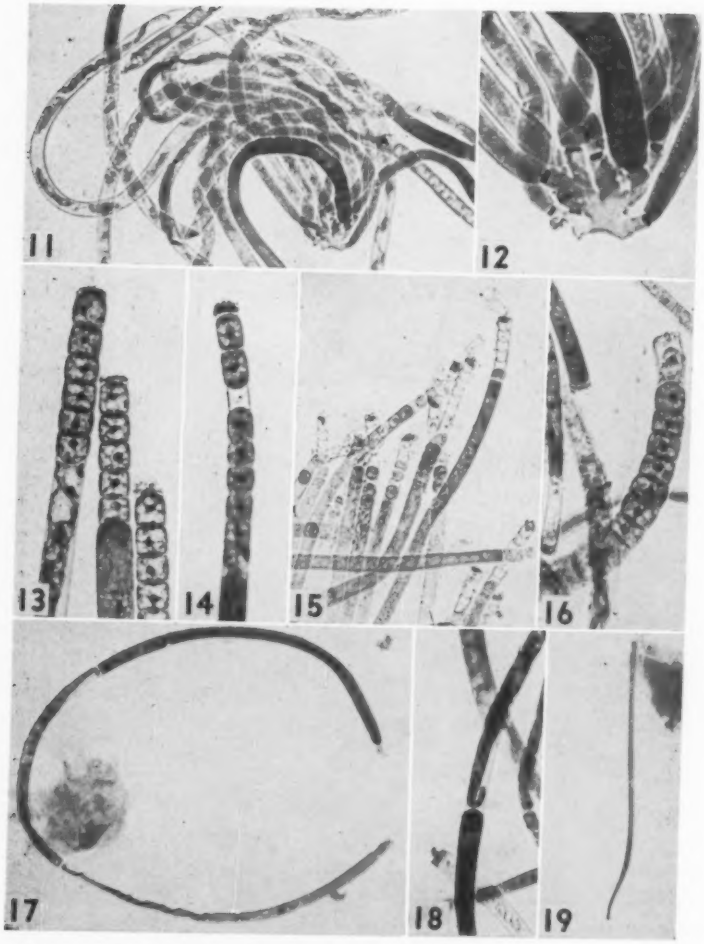
The free end of the wider filaments in the clusters mentioned above are usually found in the process of producing spores (FIGS. 13–16). These are uninucleate sporangiospores, measuring between 10–22 μ long by 9–15 μ wide. The narrower spores are usually longer, while wider spores are usually shorter; if isodiametric, they are frequently 13 $\times$ 13 μ. Nuclei within these spores, as within the hyphae, have an approximate diameter of 3–4 μ, though occasional larger nuclei have been observed.

In some hosts a noticeable number of uninucleate sporangiospores may be found in an aborted condition (FIGS. 14, 15). Empty sporangia ordinarily do not accumulate at the tips of hyphae, but only a crown of bacteria remains where the sporangial wall has broken off or decomposed (FIGS. 13, 14). Occasional hosts, upon dissection, have yielded hyphae with rows of half a dozen or more empty sporangia still remaining attached to the hyphae (FIG. 15).

Prior to emergence the spores tend to assume a spherical shape if not compressed by adjacent spores (FIG. 15). The spore walls are very thin at this time, in fact, almost like a membrane. The spores emerge most commonly through a hole or tear at the terminal end of the sporangium (FIG. 16).

Another spore type produced by these hyphae is an elongate, multinucleate sporangiospore (FIGS. 17–19) similar to that previously described as "spore type A" (Lichtwardt, **1954**). These spores have not been observed very frequently and apparently are produced by the narrower hyphae which are present in some of the clusters. The proximal tip of the spore often is found, while still in the sporangium, with a short, hook-like bend (FIGS. 17, 18). As far as can be determined, the multinucleate spores emerge from the terminal end of the sporangium, rather than from a lateral pore as do typical type A spores.

On the basis of these studies the following technical description is proposed for the species:



FIGS. 11–19. Anterior hyphae of *Enterobryus attenuatus*. 11. Lateral view of cluster of hyphae with fused holdfasts, × 200. 12. Holdfasts of Fig. 11 enlarged, × 500. 13. Uninucleate sporangiospores, × 300. 14. Aborted spore, × 300. 15. Hyphae with rows of empty terminal sporangia, × 150. 16. Spore emerging through hole in apical wall of sporangium and passing into empty terminal sporangium, × 300. 17. Hypha with elongate, multinucleate sporangiospores, × 180. 18. Bent proximal tip of multinucleate spore in a sporangium, × 360. 19. Multinucleate sporangiospore, × 180.

ENTEROBRYUS ATTENUATUS Leidy

Attached to the lining of hindgut of the beetle *Passalus cornutus* Fab. Hyphae attached to the colon filiform, sinuous near the base or essentially straight, growing singly or in tufts, up to 2 mm or slightly more in length, width about 11–12 μ near base, 15 μ at widest point, narrowing to 6–7.5 μ just back of tip. Hyphae inset in holdfast which is up to 50–70 μ long by about 17 μ wide and may show longitudinal striations. Holdfasts which are in tufts may be fused. Hyphae attached to the ileum growing singly or more commonly as clusters of recurved hyphae attached to a fused holdfast system; measuring up to 1.4 mm in length by 8–16 μ wide; producing uninucleate sporangiospores 10–22 μ long by 9–15 μ wide, or multinucleate sporangiospores about 240–250 $\times$ 5–10 μ.

DISCUSSION

One of the questions which comes to mind when one first observes both types of hyphae from the two regions of the Passalid beetle hindgut is whether or not these are separate species of fungi within the same host. The thalli of the two types certainly differ from each other in many respects more than may thalli of different species of Eccrinales from different hosts. Experimental evidence is wanting to prove whether or not we are dealing with a single species; such evidence would be difficult but not impossible to obtain. However, the observational evidence on hand is such that it convinces me that these two types of hyphae are members of the same species. Supporting the hypothesis that both types of hyphae represent but one species are the following facts:

1. Hyphae of both types have always been found by me within the same host.

2. Both types of hyphae exhibit the rather unique habit of growing in clusters, and to such an extent that the holdfasts may be fused.

3. The sigmoid posterior hyphae, which Leidy observed, rarely have been seen producing spores, although such hyphae have been found very abundant in some hosts. It seems reasonable to expect that the life cycle of this species involves a stage which produces spores more frequently, since all other species of Eccrinales, whose descriptions are based upon more than a few individual hosts, are known to produce spores of at least one type in relative abundance.

4. The elongate, multinucleate sporangiospores produced by some of the anterior hyphae show a decided similarity to young posterior hyphae (compare FIGS. 2 and 19), and are believed to give rise to these hyphae in the colon.

It appears, then, that some, if not all, of the sigmoid posterior hyphae grow from the multinucleate sporangiospores produced in the ileum. The posterior hyphae probably produce a limited number of spores, as evidenced by occasional hyphae with truncate tips and by Leidy's findings of a few hyphae with crosswalls near the apices, though it is not known whether such spores would function to propagate the fungus within the same host or to infect other individuals.

The initial infection of a host possibly occurs through ingestion of the uninucleate spores excreted in the feces of another host. I have not found newly-infected Passalid beetles among the specimens studied to check this suggestion, but in another species of *Enterobryus* from a milliped (description in press), which produces similar spores, the spores are known to form heavier walls after leaving the sporangium, which might serve as protection during their passage from one host to another. It is believed, in brief, that the uninucleate spores function mainly to infect new hosts while the multinucleate spores function mainly to multiply the fungus within the same individual.

The life cycle of species of Eccrinales living in passalid beetles may be more complex than suggested by the observations reported in this paper. *Passalus cornutus* appears to be the only common species of Passalidae in the United States east of the Rocky Mountains, and most other species are tropical. During part of 1954 and 1955 I collected specimens of tropical Passalidae from various sites in Panama, Trinidad, and Brazil, and preliminary studies revealed not only hyphae similar to those of *Enterobryus attenuatus,* but also instances of infection of the beetle larvae and hyphae in the rectum of adults, infections which have not been found present in *Passalus cornutus.* This new material will be compared with *E. attenuatus* in a future paper.

The systematic treatment of *Enterobryus attenuatus* in the literature leaves much to be desired. In **1916** Léger and Duboscq described two species of Eccrinales from Hydrophilid beetles, placing them in the genus *Eccrinopsis* which previously had been established (Léger & Duboscq, **1906**) for a species of Eccrinales found in an isopod. They came to the provisional conclusion that Leidy's species of Eccrinales from the Passalid beetle belonged in their genus *Eccrinopsis* (Léger & Duboscq, **1916**, p. 30, translated):

"Since the genus *Enterobryus* has for its type *Enterobryus elegans* Leidy from *Spirobolus marginatus* Say, it is prudent to apply it only to the parasites of diplopods. We therefore place *Enterobryus attenuatus* Leidy from *Passalus cornutus* in the genus *Eccrinopsis.* The characteristics indicated by Leidy (shape, color, structure of the holdfast

and the thallus, small number of microconidia) are found in the genus *Eccrinopsis* of the hydrophilids."

This reasoning cannot be accepted, since if generic similarities exist between *Enterobryus attenuatus* and species of *Eccrinopsis,* the latter would be placed of necessity in the genus *Enterobryus* on the basis of priority, and not vice-versa. *Enterobryus attenuatus* is separated from other species of *Enterobryus* in the above statement solely on host differences.

The separation of genera according to the type of host was carried out further by Léger and Duboscq in **1929** when they decided for morphological reasons to retain the genus *Eccrinopsis* for species of Eccrinales from Isopods, and established a new genus, *Trichella,* to include species of Eccrinales from coleopterous insects. Accordingly, they transferred *Enterobryus attenuatus* to this new genus, and it became known as *Trichella attenuata.* Likewise, a species of Eccrinales which Thaxter (**1920**) had described from an undetermined species of *Passalus* from Dominica, B. W. I., and which he had named *Enterobryus compressa,* was also placed in the genus *Trichella.*

I see no reason to transfer *Enterobryus attenuatus* either to the genus *Eccrinopsis* or to the genus *Trichella,* except on the basis of host differences. Such a separation may be convenient, but is not based upon morphological relationships. There are, it is admitted, differences between *Enterobryus attenuatus* and other species of the genus from millipeds, though the differences are not clear enough at this time to determine whether or not a generic distinction is warranted for those species from passalid beetles.

SUMMARY

New forms of hyphae from the anterior region of the hindgut of the beetle *Passalus cornutus* Fab. are described, and are believed to be another phase of the commensal fungus *Enterobryus attenuatus* Leidy, also found in the same host. A revised description for *Enterobryus attenuatus* is presented, and the taxonomy of the species is discussed.

DEPARTMENT OF BOTANY AND PLANT PATHOLOGY
IOWA STATE COLLEGE
AMES, IOWA

LITERATURE CITED

Léger, L. and O. Duboscq. 1906. L'évolution des *Eccrina* des *Glomeris.* C. R. Acad. Sci. **142**: 590–592.

——. 1916. Sur les Eccrinides des Hydrophilides. Arch. Zool. Exp. et Gén. (Notes et Revue 2) **56**: 21–31.

——. 1929. *Eccrinoïdes Henneguyi* n. g. n. sp. et la systématique des Eccrinides. Arch. Anat. Microsc. **25**: 309–324.

Leidy, J. 1849a. *Enterobrus*, a new genus of Confervaceae. Proc. Acad. Nat. Sci. Phila. **4**: 225–227.

——. 1849b. Descriptions (accompanied by drawings) of new genera and species of Entophyta. Proc. Acad. Nat. Sci. Phila. **4**: 249–250.

——. 1850a. (Observations upon an entophytic forest.) Proc. Acad. Nat. Sci. Phila. **5**: 8–9.

——. 1850b. Descriptions of new Entophyta growing within animals. Proc. Acad. Nat. Sci. Phila. **5**: 35–36.

——. 1853. A flora and fauna within living animals. Smithsonian Contributions to Knowledge **5**: 1–67.

Lichtwardt, R. W. 1954. Three species of Eccrinales inhabiting the hindguts of millipeds, with comments on the Eccrinids as a group. Mycologia **46**: 564–585.

Thaxter, R. 1920. Second note on certain peculiar fungus-parasites of living insects. Bot. Gazette **69**: 1–26.

TAXONOMY OF MARINE PYRENOMYCETES [1]

SAMUEL P. MEYERS [2]

(WITH 29 FIGURES)

Marine fungi have been reported from oceanic areas in different parts of the world for the past 100 years, and appear to comprise a definite and significant part of the microflora of the sea. This paper is concerned with the pyrenomycetous fungi of the Ascomycetes, a group well represented in the sea. A thorough examination of the systematics of these fungi has been lacking, and this present study endeavors to present a compilation and discussion of the available taxonomic literature dealing with this group.

The possible significance of Pyrenomycetes, as well as Phycomycetes and Fungi Imperfecti, in the biology and economy of marine environments has been emphasized by various workers (Zobell, **1946**; Meyers, **1953**, **1954**; Höhnk, **1954**, **1955**; Ritchie, **1954**), and further elaboration on this subject is beyond the scope of this taxonomic treatment. However, as saprophytes and parasites, these fungi may contribute to the decay of algae and other plant remains, the deterioration of wood and cordage, and the infection of marine plants and animals.

The literature on marine Pyrenomycetes is not extensive, and the earlier descriptions of these species primarily involved occasional collections that resulted largely from a study of the marine host plant. An early summarization by Cotton (**1907**) of the literature dealing with marine Pyrenomycetes listed 10 species that had been described from oceanic areas. However, several additional marine pyrenomycetous species that had been reported prior to 1907 were overlooked in this summary. Sutherland, in a series of papers, **1915**, a–c, **1916**, described 14 pyrenomycetous species, including several new genera, occurring on marine algae in the Orkney Islands and other parts of the British Isles. The work by Sutherland was the first attempt to study marine fungi as an integral component of the microflora of a particular locality.

These Pyrenomycetes did not receive further attention, other than

[1] A portion of a thesis submitted in partial fulfillment of the requirements for the degree of Doctor Philosophy, Columbia University.

[2] Present address: Marine Laboratory, University of Miami, Coral Gables, Florida.

that represented by several additional papers describing new species, until the investigations of Barghoorn and Linder in **1944**. The only exception to the paucity of literature during this period was the paper by Bauch (**1936**), in which he listed the species of marine Pyrenomycetes known up until that time. The studies of Barghoorn and Linder (**1944**) showed the occurrence of a typically marine pyrenomycetous flora with adaptations to aquatic existence through various types of spore appendages and spore dispersal mechanisms. The early breakdown of the ascus wall following ascospore formation is characteristic. These features have been discussed at length by Linder and have been mentioned in my previous studies. Barghoorn and Linder were the first to investigate systematically the marine fungal flora of wood. They showed the regular association of these fungi with submerged wood and discussed their significance in the decomposition processes involved.

Various contemporary investigators have contributed to our knowledge of marine Pyrenomycetes occurring in different areas of the world. Among these investigators are: Meyers (**1953**, **1954**), Biscayne Bay, Florida, Gulf of Mexico, Caribbean and the Bahamas; Wilson (**1951**, **1954**), Wales, Northern Ireland; Höhnk (**1954**, **1955**), Bremerhaven, North Sea; Johnson (**1956**, **1957**), Beaufort, North Carolina; Cribb *et al.* (**1955**, **1956**), Australia. These workers isolated new species and genera of these fungi and recorded new areas of occurrence for genera already described. Many of the Pyrenomycetes discussed here have diverse ecological patterns, some genera being world-wide in occurrence.

Thirty genera and approximately 70 species of marine Pyrenomycetes have been reported on a wide range of substrates, including various algae, vascular plants, wood and cordage. Of these fungi, 14 genera have been found only in marine areas, while the remaining 16 were described originally from terrestrial habitats.

In our studies, the term "marine" refers to those pyrenomycetous fungi that have been collected from sea water areas, regardless of the salinity or range of salinities of that particular area. We have used several criteria separately or together to indicate the adaptation of these fungi to a marine environment. Some of these criteria are: (1) the continuous and regular occurrence of these fungi in oceanic areas; (2) their association with marine algae and higher plants, sometimes as parasites; (3) morphological adaptation and various growth responses in culture. These characteristics are discussed under the various genera concerned.

Extreme variability in the structure of the perithecium has been observed in seven genera, viz., *Lulworthia, Ceriosporopsis, Lignincola,*

Antennospora, Arenariomyces, Peritrichospora and *Torpedospora*. Species of these genera have been studied in pure culture on a variety of different nutritional media and perithecial production has been obtained in all of the species examined.

The questionable value of perithecial characteristics as dependable taxonomic criteria has necessitated the use of the ascospore for the separation of species and genera. This latter character has been used in the key to the genera of Pyrenomycetes as well as in the keys to the species of certain of these genera. Pyrenomycetous taxa of doubtful or uncertain affinity are absent from the generic key, but are relegated to a separate section.

The presence of noticeable morphological variation within these genera suggests that present standards of taxonomic criteria used in classifying non-aquatic Pyrenomycetes may not be usable in establishing species and genera in this marine group.

METHODS

The collection and isolation methods used to study the marine Pyrenomycetes in Biscayne Bay, Florida, are described in detail in two previous papers (Meyers, **1953**, **1954**). The fungal species described from this area as well as the nearby Gulf of Mexico, Caribbean and Bahamas were collected primarily on yellow pine panels. Driftwood and wood from inshore installations such as piers and pilings also were examined for the presence of these fungi. The wood panels were submerged continuously for varying periods of from 6 to 22 weeks. A survey of the Pyrenomycetes of Biscayne Bay, Florida, showed that the intensity of fungal activity within that area varied greatly in different parts of the Bay. One species, *Antennospora caribbea* (Form No. 2) often appeared on wood within 6 weeks following submergence, while another common species, *Arenariomyces salina* (Form No. 7), was isolated from wood usually after a submergence period of from 18 to 22 weeks.

Lulworthia grandispora, L. floridana, L. medusa var. *biscaynia, Antennospora caribbea, Ceriosporopsis halima, Lignincola laevis, Torpedospora radiata, Peritrichospora integra* and *Arenariomyces salina* have been studied in pure culture, with fruiting occurring from single-spore inoculum in all of these species. These fungi correspond respectively to the Forms Nos. 1–7 discussed previously (Meyers, **1953**, **1954**). The results of these studies, including growth response to various nutritional materials and conditions favoring perithecial production, are to be presented in a subsequent paper.

Excellent vegetative growth by the above species has been maintained on a medium consisting of 0.1% Difco yeast extract, 1.0% glucose, 1.5% Bacto agar made up in sea water. Powdered cellulose, mannitol and other sugars have been substituted successfully for the glucose.

Microtome sections of young and maturing perithecia of *Lulworthia floridana, Ceriosporopsis halima, Torpedospora radiata,* and *Peritrichospora integra* were made from material in fresh, actively growing cultures. In *Antennospora caribbea, Lignincola laevis* and *Arenariomyces salina,* the young perithecia were taken from infected balsa wood maintained in separate tanks of sea water. By the use of such unifungal inoculations, it was possible to establish definitely the origin of the immature perithecium, and subsequently to determine whether the structure was a true perithecium or a pseudoperithecium.

The perithecia were killed and fixed in FAA, followed by a dehydration series of butyl alcohol. Longitudinal sections of the perithecia were cut at 7 and 10 μ. Satisfactory staining of the material was obtained by using Flemming's triple stain. Permanent slides of these perithecial sections have been prepared.

Type material of the marine species described has been placed in the Cryptogamic Herbarium, N. Y. Botanical Garden and in the Farlow Herbarium, Harvard University. Cultures of the various marine Pyrenomycetes collected during these studies have been placed in the Mycology Culture Collection of the Marine Laboratory, University of Miami, Coral Gables, Florida.

Sincere appreciation is expressed to Dr. L. S. Olive for his invaluable council and guidance during the progress of this study. Dr. D. P. Rogers kindly prepared the Latin diagnoses and generously assisted in many of the taxonomic problems. I am indebted to Dr. T. W. Johnson, Jr., who supplied the manuscripts of his several papers on marine Pyrenomycetes, and to Dr. I. Mackenzie Lamb, for making available the type specimens of the late Dr. D. H. Linder, in addition to supplying various notes and references on marine fungi. Further appreciation is extended to my wife, Gertrude K. Meyers, for her encouragement throughout this work, and for her careful preparation of the final manuscript.

The earlier phases of this investigation were done at the Marine Laboratory, University of Miami, and were aided by a contract Nonr 840 (01) between the Office of Naval Research, Department of the Navy, and the University of Miami. The extensive cooperation of those people who assisted materially in these distributional studies is acknowledged.

KEY TO GENERA OF MARINE PYRENOMYCETES[1]

1. Stroma present, clypeus-like....2
1. Stroma absent....4
2. Spores 1-celled....PHYLLACHORELLA 480
2. Spores 2-celled....3
3. Spores hyaline....PLACOSTROMA 480
3. Spores brown....DIDYMOSPHAERIA 481
4. Perithecia hemispherical or hysterioid....5
4. Perithecia spherical or flask-shaped....6
5. Perithecia hemispherical, opening by a pore....*MYCAUREOLA 482
5. Perithecia hysterioid, opening by a longitudinal slit....HYPODERMA 482
6. Ascospores spherical, ellipsoid or fusiform....7
6. Ascospores filamentous....27
7. Ascospores non-appendaged (see also LENTESCOSPORA under 21)....8
7. Ascospores appendaged (see also SAMAROSPORELLA under 10)....20
8. Spores hyaline....9
8. Spores yellowish to brown at maturity....15
9. Spores 1-celled, ellipsoid....10
9. Spores more than 1-celled....11
10. Spores encased within irregular hyaline envelope...*SAMAROSPORELLA 483
10. Spores not encased as above....GUIGNARDIA 483
11. Spores 2-celled....12
11. Spores 4(–6)-celled....14
12. Paraphyses present....MELANOPSAMMA 484
12. Paraphyses absent....13
13. Perithecia long-rostrate....*LIGNINCOLA 487
13. Perithecia not long-rostrate....MYCOSPHAERELLA 488
14. Paraphyses present....METASPHAERIA 489
14. Paraphyses absent....SPHAERULINA 490
15. Spores simple or transversely septate....16
15. Spores muriform....PLEOSPORA 490
16. Spores 1-celled....ROSELLINIA 492
16. Spores more than 1-celled....17
17. Spores 2-celled....18
17. Spores 2–4(–8)-celled....19
18. Paraphyses present....MASSARIELLA 492
18. Paraphyses absent....AMPHISPHAERIA 492
19. Perithecia soft and white with elongated neck....*ORCADIA 493
19. Perithecia dark with short black papilliform neck....*LEPTOSPHAERIA 494
20. Spores with terminal appendages only....21
20. Spores with both terminal and medial appendages, 1–3(–5)-septate....26
21. Appendages consisting of inconspicuous gelatinized terminal thickenings of the cell wall, spores 1–2(–3)-celled....*LENTESCOSPORA 495

[1] Asterisk indicates genera reported only from marine environment.

LIST OF GENERA AND SPECIES

PHYLLACHORELLA Syd.

P. OCEANICA Ferd. & Winge, Mycologia **12**: 103, f. 1, 2. 1920.

Hab. *Sargassum* sp., Atlantic Ocean.

This species and those of the following genus are the only reported marine representatives of the Dothideales.

PLACOSTROMA Th. & Syd.

P. pelvetiae (Suth.) comb. nov.
Dothidella pelvetiae Suth. Brit. Myc. Soc. Trans. **5**: 154. 1915.

Hab. *Pelvetia canaliculata,* Orkney Islands.

P. laminariae (Rostr.) comb. nov.
Dothidella laminariae Rostr. Bot. Tidsskr. **19**: 213, f. 3, 1–3. 1895.
Endodothella laminariae (Rostr.) Th. & Syd. Ann. Myc. **13**: 431. 1915.

Hab. *Laminaria longicruris,* Greenland and *Laminaria agardhii,* Jan Mayen Island, Arctic Sea (Bot. Tidsskr. **21**: 28. 1897).

The two species of *Placostroma* can be separated as follows:

	P. pelvetiae	*P. laminariae*
Locules	100–120 × 80–90μ	120–140μ
Asci	40–45 × 12–15μ	65–70 × 12–14μ
Ascospores	12.5–15 × 5–6μ (ovoid or oblong)	20–21 × 7–8μ (ellipsoid to fusiform)

Sutherland separates *P. pelvetiae* from *P. laminariae* on the basis of the smaller spores present in the former species. Sutherland reports the presence of paraphyses in *D. pelvetiae*, and although Rostrup does not mention them in his description of *D. laminariae*, they are shown clearly in his Fig. 1. Since paraphyses are present in both of these species, they cannot belong to *Dothidella* Speg., in which genus paraphyses are absent. Theissen and Sydow (Ann. Myc. **13**: 582. 1915) transferred *D. laminariae* to the paraphysate genus *Endodothella*, and later (Ann. Myc. **16**: 31. 1918) placed the species in the Pseudosphaeriales. A comparison of the description of *Endodothella* Th. & Syd. with that of the genus *Plactostroma* Th. & Syd. (Ann. Myc. **12**: 269. 1914) indicates that these two genera are synonymous. This synonymy is noted also by Clements and Shear. Since the latter genus appears to have priority over *Endodothella*, the species *D. pelvetiae* and *D. laminariae* are placed in the genus *Placostroma* Th. & Syd.

DIDYMOSPHAERIA Fckl.

D. FUCICOLA Suth. New Phytol. **14**: 188. 1915.

Hab. *Fucus vesiculosus*, Orkney Islands.

D. PELVETIANA Suth. New Phytol. **14**: 185, f. 2, 1–4. 1915.

Hab. *Pelvetia canaliculata*, Orkney Islands.

D. MARITIMA (Crouan) Sacc. Syll. Fung. **1**: 703. 1882.
Sphaeria maritima Crouan, Flor. Finistère p. 27. 1867.

Hab. *Atriplex* (*Obione*) sp., Western France.

The original description of *Sphaeria maritima* by Crouan does not include the size of the asci or ascospores. The perithecia are black, subspherical, without an ostiole. The ascus is cylindrical and paraphyses are present. Ascospores are ovoid, brown, 2-celled with 2 large vacuoles.

The perithecia of *D. fucicola* and *D. pelvetiana* are globose or flask-shaped, with a small ostiole. Sutherland describes a dark outstanding "pseudoclypeus," consisting of modified host and fungal tissue, sur-

rounding the perithecium. However, in *Didymosphaeria* Fckl., no clypeus or pseudo-clypeus is present. Likewise, Clements and Shear place this genus in the group of Sphaeriales characterized by the absence of a clypeus or epistroma. Sutherland himself notes the unsatisfactory arrangement in which species with a kind of clypeus are placed in the genus *Didymosphaeria*. The exact nature of the clypeus-like structure described by Sutherland for *D. pelvetiana* and *D. fucicola* needs further investigation.

Sutherland separates these two species on the basis of differences in the size of the perithecium and ascus, and in the shape of the ascospores. The spores of *D. pelvetiana* are 18–24 × 5–6 μ while those of *D. fucicola* are 16–22 × 7–8 μ. The spores of the former species are fusiform in shape while those of the latter species have a broad upper end and a narrow tapering lower end with a constriction at the septum. The spores of both species become slightly yellowish at maturity.

*MYCAUREOLA Maire & Chemin

Type: *M. dilseae* Maire & Chemin, monotype.

M. DILSEAE Maire & Chemin, Compt. Rend. Acad. Sci. Paris **175**: 320, f. 1–4. 1922.

Hab. *Dilsea edulis*.

The perithecia of this genus are hemispherical, white, with a pore at the top at maturity. Paraphyses are present. The ascospores are spherical, 1-celled, hyaline, without appendages.

This genus, and *Trailia* Suth. and *Orcadia* Suth., are the only marine representatives reported belonging to the Hypocreales. The description of *Mycaureola* approaches that of *Hyponectria*. However, the former genus can be separated from the latter by the presence of paraphyses and by the characteristic spherical ascospores.

HYPODERMA DC. ex Gray

H. LAMINARIAE Suth. New Phytol. **14**: 190, f. 3, 1–5. 1915 (31 Mar.). See also Brit. Myc. Soc. Trans. **5**: 153. 1915 (10 May), where erroneously indicated as new.

Hab. *Laminaria saccharina*, Orkney Islands; Newcastle, Northern Ireland, 1951 (Wilson).

This species is the only representative of the Hysteriaceae that has been reported from a marine environment. The spores of *H. laminariae* develop a faint yellow color when mature, while the spores of the genus

Hypoderma DC. are typically hyaline in color. However, since the spores of *H. laminariae* are hyaline until almost maturity, and the final coloration is very light, there does not appear to be sufficient reason for removing this species from the genus *Hypoderma*.

*SAMAROSPORELLA Linder

Type: *S. pelagica* Linder, monotype.

S. PELAGICA Linder, Farlowia **1**: 408, pl. 3, f. 1–4. 1944.

Hab. Driftwood, Provincetown, Massachusetts, 1943 (Barghoorn 18).

The perithecia of this genus are globose, black and carbonaceous with a papilliform neck. Paraphyses are absent. The ascospores are elongate-ellipsoid, hyaline, with an irregular hyaline envelope forming "wings." Smaller aborted spores may occur in which these hyaline envelopes are absent or else very rudimentary.

The genus *Samarosporella*, on the basis of the hyaline sheath or "wings" of the ascospores, appears closely allied to *Samarospora* Rostrup (Bot. Centralbl. Beih. **3**: 3. 1893), but differs from that genus in the ostiolate perithecia and in the fusoid rather than globose or subglobose asci.

GUIGNARDIA Viala & Rav.

1. Ascospores less than 20 μ in length.....2
1. Ascospores more than 20 μ in length.....3
2. Spores oblong to oval with rounded apices.....*G. ulvae*
2. Spores narrowly elliptical with pointed apices.....4
3. Spores less than 15 μ in diameter.....*G. irritans*
3. Spores more than 15 μ in diameter.....*G. tumefaciens*
4. Asci less than 50 μ in length.....*G. alaskana*
4. Asci more than 50 μ in length.....*G. prasiolae*

G. ALASKANA Reed, Univ. Calif. Pub. Bot. **1**: 161, pl. 16, f. 14. 1902.

Hab. *Prasiola borealis*, Alaska, Kodiak Island.

G. PRASIOLAE (Wint.) Lemm. Abh. Nat. Ver. Bremen **17**: 199. 1901. *Laestadia prasiolae* Winter, Hedw. **26**: 16. 1887.

Hab. *Prasiola tessellata*, Kerguelen's Land, Tierra del Fuego, Antarctic.

G. ULVAE Reed, Univ. Calif. Pub. Bot. **1**: 160, pl. 15, f. 1–6. 1902.

Hab. *Ulva californica*, San Francisco Bay, California.

G. IRRITANS Setch. & Estee, Univ. Calif. Pub. Bot. **4**: 311, pl. 35, f. 3–6. 1913.

Hab. *Cysteoseira osmundacea* and *Halidrys dioica,* San Pedro, California.

G. TUMEFACIENS Cribb & Herbert, Papers, Univ. Queensland Dept. Bot. **3**(2): 9, f. 1, a–d. 1954.

Hab. *Sargassum* sp., Low Head, Tasmania.

The length of the ascospores in the species *G. ulvae, G. alaskana* and *G. prasiolae* is quite similar. In *G. ulvae* the spores are 10–13 × 3.5–7 μ, in *G. alaskana,* 8.5–13.5 × 3–4 μ, and in *G. prasiolae,* 12–15 × 3.5–4.5 μ.

Reed discusses the fungus-host relationship with reference to the symbiotic or parasitic association of *G. ulvae* and *G. prasiolae* with their respective algal hosts. In *G. ulvae,* constant association of the fungus with the *Ulva* plant seems apparent, since all specimens of *Ulva californica* examined by Reed showed mycelial infection as well as perithecial production by this fungus. The relationship of *G. prasiolae* to *Prasiola borealis* is similar to that mentioned above for *G. ulvae,* although in the former, the association appears to be more lichen-like. Reed describes these algal-fungal associations as "composites," closely related to and approaching the true lichen structure. To date, no marine species of this genus have been reported from substrates other than algae. The lichen-like relationship of the fungus and alga mentioned above warrants a re-examination of some of these species to determine if this association is actually that of a true lichen.

A marine species, *G. gloeopeltidis* Miyabe & Tokida, incorrectly described in the genus *Guignardia,* is discussed in the list of doubtful genera and species at the end of this section.

MELANOPSAMMA Niessl

M. TREGOUBOVII (Oll.) Oll. Inst. Oceanographique Ann. II **7**: 172, pl. 1, f. 1–10. 1929.

Thallassoascus Tregoubovii Oll. Compt. Rend. Acad. Sci. (Paris) **182**: 1348. 1926.

Hab. *Zanardinia collaris, Aglaozonia chilosa, A. parvula* and *Cystoseira abrotonifolia,* Nice, Southern France; *Cystoseira abrotanifolia,* Carthage, Tunisia; Cherchel, Algeria; Banyuls, France, 1931 (Feldmann, J. Rev. Algol. **6**: 225–226. 1932); *Dilophus fasciola,* Dinard, France, 1952 (Aleem, A. A. Bull. Lab. Dinard **36**: 21–25, f. 1–4. 1952).

M. balani (Winter) comb. nov.

Epicymatia balani Winter, Jour. de Bot. **1**: 233. 1887.

Pharcidia balani (Winter) Bauch, Publ. Sta. Zool. Napoli **15**: 379. 1936.

Hab. *Brachytrichia balani,* St. Malo, France.

M. cystophorae (Cribb & Herbert) comb. nov.

Otthiella cystophorae Cribb & Herbert, Papers, Univ. Queensland Dept. Bot. **3**(2): 10, f. 2, a–c. 1954.

Hab. *Cystophora retraflexa,* Port Arthur, Tasmania.

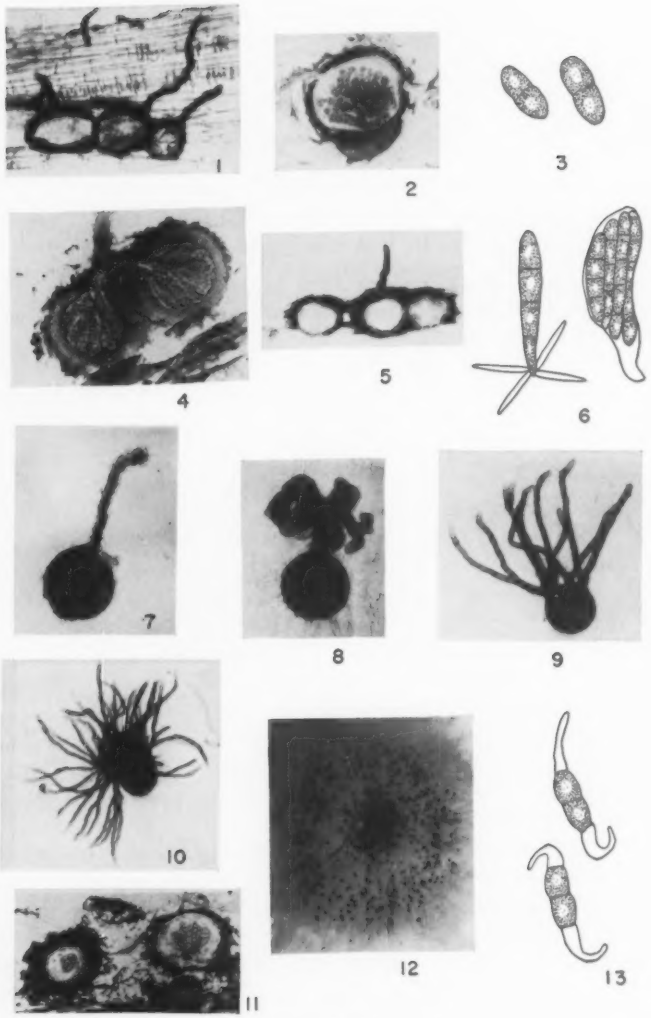
The genus *Melanopsamma* Niessl is closely related to *Pharcidia* Korb., and is separated by Clements and Shear on the premise that *Pharcidia* is lichenicole while *Melanopsamma* is not lichenicole. Since *Pharcidia balani* does not occur on lichens, a more correct disposition of this species would probably be in the genus *Melanopsamma.*

The genus *Otthiella* Sacc. is very similar to both *Pharcidia* and *Melanopsamma.* The primary distinguishing feature used by Clements and Shear in separating these genera is the occurrence of innate or finally erumpent perithecia in *Otthiella.* The perithecia of *Pharcidia* and *Melanopsamma* are mentioned by Clements and Shear as being superficial. However, the description of the genus *Pharcidia* (Saccardo **9**: 676) notes the perithecia as partly immersed or becoming emergent. In the marine species of *Pharcidia,* the perithecia are immersed. From these conflicting descriptions, it appears that the character of superficial or innate perithecia cannot be used to separate these marine species of *Pharcidia* and *Otthiella.* The similarity in ascospore and perithecial characteristics of the marine species of *Pharcidia, Otthiella* and *Melanopsamma* warrants the inclusion of species of the two former genera in the single genus *Melanopsamma.*

The perithecia of all three species of *Melanopsamma* listed above are globose to subglobose, membranous to carbonaceous and opening by an apical pore. In *M. balani,* the perithecia are 120–140 μ, in *M. cystophorae,* up to 1000 μ in diameter, and in *M. Tregoubovii,* 500–750 μ. The characteristics of the ascospores of these three species are as follows:

	Shape	Size
M. balani	oblong-subclavate	19–23 × 6–7μ
M. cystophorae	ellipsoid to cylindrico-ellipsoid	50–60 × 15–21μ
M. Tregoubovii	ellipsoid (rounded apices)	*40–45 × 28–30μ

* Aleem found the ascospores of *M. Tregoubovii* on *Dilophus fasciola* to be 25–30 × 15–20μ. However, he noted that this might be due to the long preservation of the material in alcohol.



FIGS. 1–13.

FIGS. 1–3. *Lignincola laevis*. 1. Section of infected wood with imbedded ostiolate perithecia, × 48. 2. Longitudinal section of young perithecium, × 165. 3. Ascospores, × 360. FIGS. 4–6. *Torpedospora radiata*. 4. Longitudinal section of young adjacent perithecia, × 90. 5. Section of infected wood with non-necked and

Ollivier divided *M. Tregouboxii* into two varieties, var. *cutleriae* on *Zanardinia* and *Aglaozonia* and var. *cystoseirae* on *Cystoseira*. He observed that the asci and ascospores of var. *cystoseirae* are larger than those of var. *cutleriae*. Another distinguishing feature of var. *cutleriae* is the presence of a thick stipe, 500–750 × 200–300 μ, attaching the perithecium to the substrate.

Two other species, *Pharcidia pelvetiae* Suth. and *Pharcidia marina* Bommer, apparently do not belong in this genus, and are discussed in the list of doubtful genera and species.

*LIGNINCOLA Höhnk

Type: *L. laevis* Höhnk, monotype.

L. LAEVIS Höhnk, Veröff. Inst. Meeresforsch. Bremerhaven **3**(2): 216, pl. 5, f. 1–4. 1955.

FIGS. 1–3

Hab. Wood, including yellow pine, Bremerhaven, Germany, 1952–1953 (Höhnk); Biscayne Bay, Florida, 1953–1954, Cedar Key, Florida, January, 1954, and Grand Cayman, Cayman Islands, January, 1954 (Meyers).

The perithecia of *Lignincola* are globose to elongate, membranous, light to dark fuscous with a characteristically long neck. The neck is terminal or central, straight or curved and occasionally branched and irregular. Paraphyses are absent. Asci are fusiform or clavate with the ascus wall retained at maturity. Ascospores are ellipsoid with rounded apices, 2-celled, hyaline, non-appendaged and with one or several large vacuoles in each cell.

The genus *Lignincola* is similar to Form No. 4 (Meyers, **1954**). The ascospores of *L. laevis* are 16.2–20.3 × 6–8.1 μ, while those of Form No. 4 are 21–23 × 7–8 μ. Although the spores of Form No. 4 are slightly longer than are those of *L. laevis*, this difference in size does not appear sufficient to warrant the establishment of a new species.

Several features of this genus observed in the present study, and not reported by Höhnk, are incorporated into the above discussion. Whereas

long-necked perithecia, × 33. 6. Ascus, × 150, and ascospores, × 475. FIGS. 7–13. *Ceriosporopsis halima*. 7. Single-necked perithecium, × 47. 8. Perithecium with extreme malformation of the neck, × 43. 9. Multi-necked perithecium, × 33. 10. Perithecium with vigorous neck production and merging of venters of several perithecia, × 30. 11. Longitudinal section of young perithecia. Perithecium at right shows developing ascogenous system, × 80. 12. Colony growth with extensive production of perithecia. 13. Ascospores, × 277.

Höhnk reported only the presence of globose perithecia with apical necks, we have noted both globose and elongate perithecia with central as well as terminal necks. The latter may be irregular and branched and there may occasionally be more than a single neck on a perithecium. The characteristic long cylindrical neck of the perithecium has been observed both in field collections of wood and in laboratory studies of balsa wood sections infected with *L. laevis*. This long neck is a diagnostic feature of the genus.

Several characteristics distinguish *Lignincola* from other Pyrenomycetes collected in Biscayne Bay, Florida. The former genus has typically non-appendaged ascospores, and as noted in previous studies, the ascus wall persists well after the spores have matured. Germination of the ascospores while still within the ascus has been observed. In those genera of Pyrenomycetes collected in Biscayne Bay, Florida, as well as in many of those described by Linder, early dissolution of the ascus wall is a common feature. From its pattern of wide oceanographic distribution and regular occurrence within the areas examined, *L. laevis* readily appears to be a normal marine inhabitant. Thus, the absence of more obvious marine features in this species, such as appendaged spores and deliquescent asci, does not indicate necessarily a lessened degree of adaptation to a sea water environment. Less than half of the Pyrenomycetes reported in this paper have appendaged spores and deliquescent asci.

Sections of perithecia of *L. laevis* have failed to show the presence of paraphyses or pseudoparaphyses. A study of this material indicates that the fruiting structure develops as a true perithecium. In this feature, *Lignincola* differs from the following genus, *Mycosphaerella,* which has pseudoperithecia and is placed in the Pseudosphaeriales (Gäumann, 1952). The ascospores of both genera are similar in appearance.

MYCOSPHAERELLA Johans.

M. ASCOPHYLLI Cotton, Brit. Myc. Soc. Trans. **3**: 96, pl. 4. 1907.

Hab. *Ascophyllum nodosum,* British Isles (English Channel, North Sea, Irish Sea), Faeroe Islands, Heligoland and Sweden (West Coast); Wales and Northern Ireland, 1951 (Wilson).

M. PELVETIAE Suth. New Phytol. **14**: 34, f. 1, 1–4. 1915.

Hab. *Pelvetia canaliculata,* British Isles; Wales and Northern Ireland, 1951 (Wilson).

Sutherland separates *M. pelvetiae* from *M. ascophylli* on the basis

of dissimilarities in size of perithecia, asci, and ascospores and in mycelial diameter. However, as indicated below, these structures of the two species are not significantly different in size. It is doubtful if the presence of larger perithecia in *M. ascophylli* is sufficiently significant to separate this species from *M. pelvetiae*. Nevertheless, the two species tentatively are treated separately here.

	M. pelvetiae	*M. ascophylli*
Perithecia	65–85μ (spherical)	100–130 × 80–90μ
Asci	45–55 × 15–20μ	50–60 × 18–20μ
Ascospores	19–25 × 4.5–5.5μ	18–21 × 4–5μ
Mycelium (diam.)	1.0–1.25μ	1.5–2.0μ

In both *M. ascophylli* and *M. pelvetiae*, the perithecia are confined to the receptacles of the respective algal host. Sutherland observed the continuous association of *M. pelvetiae* with the alga *P. canaliculata*, in all plants of the latter examined, and suggested a possible symbiotic relationship between the two. Smith and Ramsbottom (New Phytol. **14**: 295–298. 1915) discuss the relationship of *Mycosphaerella pelvetiae* to its algal host, *Pelvetia canaliculata*. They mention the possible similarity of the fungal-algal association to a lichen-like structure, and suggest a further study of the nature and extent of the possible symbiosis involved. It is pointed out that the vegetative and reproductive structure of the *Pelvetia-Mycosphaerella* complex is totally different from anything known in lichens.

METASPHAERIA

M. AUSTRALIENSIS Cribb & Cribb, Papers, Univ. Queensland Dept. Bot. **3**(10): 79, f. 1, a–d. 1955.

Hab. *Avicennia marina* var. *resinifera*, Redcliffe, Australia.

This genus is closely related to *Ceratosphaeria* which has yellow or yellow-brown spores, while *Metasphaeria* includes those leptosphaeriaceous species with hyaline spores. Unfortunately, Cribb and Cribb fail to give the color of the ascospores in their description of *M. australiensis*. However, the spore wall of this species is constricted at the septa while those of the genus *Ceratosphaeria* are not constricted. On the basis of the perithecial type alone, *M. australiensis*, with long-necked perithecia, would appear more closely allied to the genus *Ceratosphaeria*. Clements and Shear place the latter genus among those fungi which have perithecia with necks, while *Metasphaeria* is placed in the group lacking necks. Thus, the occurrence of *M. australiensis*, a species with necks, in a genus typically without necks, is further evidence of the questionable

value of the presence or absence of the perithecial neck as a criterion in specific or generic separation.

SPHAERULINA Sacc.

S. ORAE-MARIS Linder, Farlowia **1**: 413, pl. 4, f. 12–15. 1944.

Hab. Wood, Long Beach, California, 1943 (Dethier).

S. PEDICELLATA Johnson, Mycologia **48**: 846. [1956] 1957.

Hab. Pine panel, Beaufort, North Carolina, 1955.

S. sp. (Johnson, Mycologia **48**: 847. [1956] 1957).

Hab. Pine panel, Beaufort, North Carolina, 1955.

The size of the perithecia, asci and ascospores of these three species is given below.

	Perithecia	Asci	Ascospores Size	Septa
S. orae-maris	200μ diam.	72 × 10–14μ	26–30 × 5–6.5μ	3
S. pedicellata	412–720 × 250–325μ	120–215 × 16–24μ	38–54 × 10–15μ (predominantly 40–51 × 11–14μ)	3–5
S. sp.	301–426 × 154–216μ	125–150 × 10–15μ	25–43 × 4–8μ (predominantly 26–31 × 4–7μ)	5

Johnson notes that the ascospores of *S. pedicellata* are elongate-ovoid to elongate-pyriform and have one end-cell characteristically attenuated and curved. In *S. orae-maris,* the ascospores are fusoid and bluntly rounded at both ends. The ascospores of *S.* sp. are quite similar in size to those of *S. orae-maris.* However, the former fungus has 5-septate spores, while the spores of the latter species are 3-septate. As apparent in the above table, the asci and perithecia of *S.* sp. are considerably larger than are those of *S. orae-maris.*

PLEOSPORA Rab.

1. Ascospores less than 35 μ in length.....*P. pelvetiae*
1. Ascospores more than 35 μ in length.....2
   2. Ascospores less than 15 μ in diameter.....*P. pelagica*
   2. Ascospores more than 15 μ in diameter.....*P. maritima*

P. LAMINARIANA Suth. Brit. Myc. Soc. Trans. **5**: 260, pl. 5, f. 8–10. 1916.

Hab. *Laminaria* sp., British Isles.

P. MARITIMA Rehm, Hedw. 149. 1896.

Hab. *Triglochin maritima,* Norway.

P. PELAGICA Johnson, Mycologia **48**: 504, f. 21–25. 1956.

Hab. *Spartina alterniflora,* Beaufort, North Carolina, 1955.

P. PELVETIAE Suth. New Phytol. **14**: 41, f. 4. 1915.

Hab. *Pelvetia canaliculata,* British Isles.

According to Vestergren (Bot. Notiser **1900**: 37), *P. maritima* is a synonym of *Pleospora dietziana* Hazl. (Math. es. term. Közlem, Budapest **25**(*2*): 154, tab. 10, f. 40. 1892), also occurring on *Triglochin maritima.* This investigator has not seen the type or collected material of these two species; hence an evaluation of possible synonymy cannot be made. It should be noted that the name *P. maritima* was used prior to that of Rehm to describe a species of *Pleospora* occurring on the foliage of *Psamma arenaria* (Syll. Fung. **9**: 893. 1891).

Johnson, in his paper describing *P. pelagica,* also discusses the other marine species of *Pleospora.* In addition to observed differences between the species in spore size, differences in septation of the spores occur. *P. pelvetiae* has 7-septate ascospores, *P. pelagica,* 7–9-septate spores, and *P. laminariana,* 5–7-septate spores. In *P. pelagica,* only one longitudinal septum is produced in any cell, whereas in *P. maritima* longitudinal septa are commonly 2–3 per cell.

The species *P. laminariana* is not listed in the above key of marine species of *Pleospora* since Sutherland does not give the ascospore dimensions of the fungus in his original description. However, Sutherland does separate *P. laminariana* from *P. pelvetiae* on the basis of differences between perithecia of the two species. The perithecia of *P. laminariana* are larger than are those of *P. pelvetiae,* and are irregularly globose and non-necked.

Sutherland does not mention paraphyses in his description of *P. pelvetiae,* and in *P. laminariana* he describes the paraphyses as being deliquescent at maturity. In the genus *Pleospora* Rab. paraphyses or paraphysoids are persistent. However, since the characteristic muriform ascospores of *P. pelvetiae* and *P. laminariana* are typical of the genus *Pleospora,* this discrepancy in paraphysate condition does not seem sufficient to warrant the transfer of these two species to another genus.

ROSELLINIA De Not.

R. LAMINARIANA Suth. Brit. Myc. Soc. Trans. **5**: 257, pl. 5, f. 1–3. 1916.

Hab. *Laminaria* sp., British Isles.

Sutherland places this species in the genus *Rosellinia* because of the habit of the perithecium, its carbonaceous and brittle nature, the persistent walls of the ostiole and the tufted hairy coating. Occasionally two necks occur on individual perithecia, with bent as well as straight necks present.

MASSARIELLA

M. MARITIMA Johnson, Mycologia **48**: 846. [1956] 1957.

Hab. Pine panel, Beaufort, North Carolina, 1955.

This species is characterized by uniseptate, yellow-brown ascospores surrounded by a gelatinous sheath. Johnson notes that *M. maritima* might equally well be placed in the genus *Massaria,* since most species of the latter genus have pluriseptate spores, or if 1-septate, they tend toward more septa. The genus *Massariella* is limited to species with uniseptate spores.

AMPHISPHAERIA Ces. & DeNot.

A. BITURBINATA (Dur. & Mont.) Sacc. Syll. Fung. **1**: 729.
Sphaeria biturbinata Dur. & Mont. Flore Algérie 497, pl. 26, f. 7. 1846.

Hab. *Posidonia oceania,* Algiers, Algeria.

A. POSIDONIAE (Dur. & Mont.) Ces. & DeNot. Soc. Crittogam, Ital. Comm. **1**: 224. 1863.
Sphaeria posidoniae Dur. & Mont. Flore Algérie 502, pl. 25, f. 8. 1846.

Hab. *Posidonia oceania* Marseilles, Toulon, France and LaGalle, Mostagonem, Algiers, Algeria; *Zostera oceanica,* Brest, France (Crouan, Flor. Finistère 28. 1867).

A. MARITIMA Linder, Farlowia **1**: 411, pl. 3, f. 13–16. 1944.

Hab. Wood, Bucksport, Maine, 1942 (Barghoorn 13); Woods Hole, Massachusetts, 1942 (Barghoorn 3a); Aberystwyth and Dale, Wales, 1951 (Wilson).

These three species can be separated on the basis of differences in size of the ascus and ascospores as well as in the shape of the ascus.

	Asci	Ascospores
A. posidoniae	elongate-cylindrical 250–300 × 20μ	50–56 × 16–17μ
A. biturbinata	sacciform-obovate 160 × 80μ	70 × *ca.* 40μ
A. maritima	cylindrical 54.5–60 × 8–13μ	16.5 × 5μ

*ORCADIA Sutherland

Type: *O. pelvetiana* Suth., LECTOTYPE (S. P. M.).

O. ASCOPHYLLI Suth. Brit. Myc. Soc. Trans. **5**: 151, pl. 3, f. 3, 1–3. 1915.

Hab. *Ascophyllum nodosum,* Orkney Islands; Aberystwyth, Wales, 1951 (Wilson).

O. PELVETIANA Suth. New Phytol. **14**: 183, f. 1, 1–4. 1915.

Hab. *Pelvetia canaliculata,* Orkney and Clare Islands.

O. sp. (Suth. Brit. Myc. Soc. Trans. **5**: *262,* pl. 5, f. 11–14. 1916).

Hab. *Fucus vesiculosus,* British Isles.

The perithecia of this genus have a long tapering neck and light-colored walls that do not darken at maturity. Paraphyses are present. Ascospores are cylindrical, 3-septate, slightly curved and hyaline. Sutherland noted that among the marine Pyrenomycetes, only in the genera *Trailia* and *Orcadia* does the neck wall remain soft and white even after reaching the surface of the substrate. Petch (Brit. Myc. Soc. Trans. **21**: 283, 286. 1938) in his study of British Hypocreales, places these two genera in the family Nectriaceae of the Hypocreales.

Sutherland separates *O. ascophylli* from *P. pelvetiana* on the basis of the smaller perithecia, asci and ascospores of the latter species. However, present comparison is not possible since Sutherland does not give the size of the ascospores in his description of *O. ascophylli.* Sutherland observed that *O. ascophylli* and *O. pelvetiana* have a distinctive distributional pattern in that both fungi appear to be restricted to their particular algal hosts. *O.* sp. resembles *O. pelvetiana* in size of asci and ascospores, but has smaller perithecia than the latter species.

LEPTOSPHAERIA Ces. & DeNot.

1. Ascospores 1–3-septate.....2
1. Ascospores 3–7-septate.....5
2. Spores less than 25 μ in length.....3
2. Spores more than 25 μ in length.....4
3. Spores 12–18 μ in length.....*L. halima*
3. Spores 18–25 μ in length.....*L. orae-maris*
4. Spores less than 35 μ in length.....*L. discors*
4. Spores more than 35 μ in length.....*L. marina*
5. Spores 3–5-septate.....*L. maritima*
5. Spores 5–7-septate.....*L. albopunctata*

L. HALIMA Johnson, Mycologia **48**: 502, f. 30–35. 1956.

Hab. Yellow poplar and *Spartina alterniflora,* Beaufort, North Carolina, 1955.

L. ORAE-MARIS Linder, Farlowia **1**: 413, pl. 4, f. 8–11. 1944.

Hab. Driftwood, Long Beach, California, 1943: driftwood and *Spartina alterniflora,* Beaufort, North Carolina, 1955 (Johnson).

L. DISCORS (Sacc. & Ell.) Sacc. & Ell. Mich. **2**: 567. 1879.
Metasphaeria discors Sacc. & Ell. Syll. Fung. **2**: 173. 1883.

Hab. *Spartina* sp., Cape May, New Jersey: driftwood and *Spartina alterniflora,* Beaufort, North Carolina, 1955 (Johnson).

L. MARINA Ell. & Ev. Jour. Mycol. **1**: 43. 1885.

Hab. *Spartina* sp., Cape May, New Jersey; *Spartina alterniflora* and *Juncus maritimus,* Beaufort, North Carolina, 1955 (Johnson).

L. MARITIMA (Cke. & Plowr.) Sacc. Syll. Fung. **2**: 73. 1883.
Sphaeria maritima Cke. & Plowr. Grevillea **5**: 120. 1877.

Hab. *Juncus maritimus; Spartina alterniflora* and *Juncus maritimus,* Beaufort, North Carolina, 1955 (Johnson).

L. ALBOPUNCTATA (West.) Sacc. Syll. Fung. **2**: 72. 1883.
Sphaeria albopunctata West. Bull. Acad. Roy. Belg. II. **7**: 87. 1859.
Sphaeria incarcerata B. & C. Grevillea **4**: 152. 1876.
Leptosphaeria incarcerata (B. & C.) Sacc. Syll. Fung. **2**: 86. 1883.
Leptosphaeria sticta Ell. & Ev. Jour. Mycol. **1**: 43. 1885.
Leptosphaeria spartinae Ell. & Ev. Jour. Mycol. **1**: 43. 1885.

Hab. *Spartina* sp., Cape May, New Jersey, and *Juncus maritimus,* Ocean Springs, Mississippi; driftwood and *Spartina alterniflora,* North Carolina, 1955 (Johnson).

L. CHONDRI (Rostr.) Rosen. Bot. Tidsskr. **27**: 33–35. 1906.
L. marina Rostr. Bot. Tidsskr. **17**: 234. 1890.

Hab. *Chondrus crispus*, Denmark; British Isles (Cotton, 1907).

Saccardo (Syll. Fung. **9**: 797) notes that the spores of *L. marina* Rostr. are yellow, 16 × 4 μ and 1–3-septate. However, this conflicts with the findings of Rosenvinge, who examined the specimens of this species and found the spores to be 29–31 × 5–6 μ, 2-celled and hyaline. In view of these conflicting descriptions, *L. chondri* is not included in the preceding key.

Johnson, in a compilation of the literature on the marine species of *Leptosphaeria*, has redescribed certain of the species as they occur in the Beaufort, North Carolina, area, in addition to describing a new species of the genus. The paper by Johnson is the first attempt to study systematically these marine leptosphaeriaceous species and to characterize differences between individual species.

Johnson notes that *L. maritima* resembles the forms of *L. albopunctata* with 5-septate spores. However, in the former species, the mature spores are surrounded by a gelatinous sheath, while those of the latter species are not. Also, the ascospores of *L. albopunctata* are prominently guttulate, whereas those of *L. maritima* are non-guttulate. The 3-septate spores of *L. marina* resemble those of *L. maritima*, but differ in that a gelatinous sheath surrounds the mature spores of *L. maritima*. The spores of *L. marina* lack this gelatinous sheath.

*LENTESCOSPORA Linder

Type: *L. submarina* Linder, monotype.

L. SUBMARINA Linder, Farlowia **1**: 411, pl. 4, f. 1–3. 1944.

Hab. Wood, Bridgeport, Connecticut, 1942 (Barghoorn 30).

The perithecia of this genus are ovoid to subcylindrical, dark brown, with a minute papillate ostiole. Paraphyses are absent. Ascospores are ellipsoid or elongate-ellipsoid, 23–28 × 6–10 μ, 0–1(–2)-septate, hyaline, with the walls of the ends of the spores thickened and somewhat gelatinized.

Linder mentions the possible close relationship of this genus to both *Ceriosporopsis* and to *Remispora*. The perithecia of all three genera are membranous and collapsing, and the spores are hyaline, 2-celled, and with modified appendages. In *Lentescospora*, the appendages are mere thickenings resulting from gelatinization of the apical walls of the spores.

***Torpedospora** gen. nov.

Perithecia superficialia vel immersa, subhyalina vel fusca, membranacea, globosa vel subglobosa, ostiolata, collo papilliformi vel exserto, centrali. Paraphyses nulli. Asci elongato-clavati, mox deliquescentes. Ascosporae elongatae, attenuatae, 4-cellulares, hyalinae, ad finem inferiorem appendiculis quaternis radiantibus ornatae.

Perithecia superficial to imbedded, subhyaline to dark, membranous, collapsing on drying, globose to subglobose, ostiolate; neck papilliform to exserted, central. Paraphyses absent. Asci elongate-clavate, early deliquescent. Ascospores elongate, tapering, 4-celled, hyaline, with radiating appendages at the lower end.

TYPE: *T. radiata* Meyers.

The genus *Torpedospora* is distinguished from other genera with appendaged spores by the characteristic torpedo-like shape of the spore, together with the radiating appendages at the basal end.

T. radiata sp. nov.

FIGS. 4–6

Perithecia superficialia vel immersa, subhyalina vel fusca, membranacea, globosa vel interdum pyriformia, 100-324 × 100–330 μ (collo excluso), collis subpapilliformibus vel elongatis, ad 160 μ long. et 19–28 μ latis, subhyalinis vel fuscis, numero formaque variis. Asci elongato-clavati, mox deliquescentes. Ascosporae elongatae, attenuatae, 31–39 × 4–5.5 μ, hyalinae, 4-cellulares, cellula quaque vacuola dua magna et plura minora continente; appendiculae acuminatae, trinae v. quaternae, 19–23 μ long, ad basem 2.5–3 μ crass., nonnumquam deliquescentes.

Perithecia superficial to imbedded, subhyaline to dark, occasionally subhyaline below, becoming dark fuscous above, membranous, globose to occasionally pyriform, 100–324 × 100–330 μ (excluding neck); necks slightly papillate to elongate, up to 160 μ in length by 19–28 μ in width, subhyaline to fuscous, variable in number and shape. Asci elongate-clavate, deliquescing very early. Ascospores elongate and tapering (club-shaped), 31–39 × 4–5.5 μ, hyaline, 4-celled, each cell with two large vacuoles and several smaller vacuoles; spore occasionally breaking into 1- or 2-celled segments; appendages slender, acuminate, 3–4 in number, 19–23 μ long and 2.5–3 μ wide at base, occasionally deliquescent.

Hab. Yellow pine panels, July, 1953, Biscayne Bay, Florida.

T. radiata is the Form No. 5 discussed by the writer in a previous paper. Sections of young perithecia of *T. radiata* do not show the presence of paraphyses or pseudoparaphyses. The ascus wall breaks down very early in the maturation of the fruiting structure, and mature spores are found scattered within the venter.

Only several perithecia of this species were observed on yellow pine wood in a long series of collections from Biscayne Bay, Florida. However, vigorous production of perithecia occurs in agar and on wood in laboratory tanks of aerated sea water.

*CERIOSPOROPSIS Linder

Type: *C. halima* Linder.

The perithecia of *Ceriosporopsis* are partly to completely immersed, becoming exposed by the erosion of the substrate, globose to elongate, light to dark fuscous, mostly membranous, collapsing on drying, ostiolate. The neck is centric to eccentric, papilliform to elongate, occasionally branched, with one to many necks on individual perithecia. Paraphyses are absent. Asci are 8-spored, elongate, fusoid, early deliquescent. Ascospores are oval to elliptical, hyaline, 2-celled, appendaged; appendages terminal, mostly stout, tapering and elongate, one at each end, occasionally dehiscent.

Wilson studied British collections of *C. halima* and emended Linder's original description of the genus to include a long perithecial neck, light-colored perithecial walls, and 8-spored asci. Linder distinguished the genus *Ceriosporopsis* from *Ceriospora* by the irregular shape of the membranous collapsing perithecia, the deliquescent asci and the deliquescent or dehiscent appendages of the ascospores in the former genus. Also, paraphyses are present in *Ceriospora* but are absent in *Ceriosporopsis*. I have found that the shape of the perithecium depends on the type of substrate on which the fungus occurs, as did Wilson in Wales. This feature is discussed further in the section dealing with variability in *C. halima*. The latter species is the Form No. 3 referred to previously (Meyers, **1954**).

C. HALIMA Linder, Farlowia **1**: 409, pl. 3, f. 10–12. 1944.
C. barbata Höhnk, Veröff. Inst. Meeresforsch., Bremerhaven **3**(2): 210, pl. 2, f. 1–5. 1955.

FIGS. 7–13

Hab. Wood, including pine, spruce, alder and Douglas fir, Portsmouth, New Hampshire, 1942 (Barghoorn 11); Newburyport, Massachusetts, 1942 (Barghoorn 19a); Saybrook, Connecticut, 1942 (Barghoorn 17, Type); Provincetown, Massachusetts, July, 1955 (Meyers); Biscayne Bay, Florida, 1953–1954 (Meyers); Port Aransas, Texas, 1954 (Meyers); Aberystwyth, Wales, 1951 (Wilson); Watwick Bay,

Pembrokeshire, Wales, 1951 (Wilson); Copenhagen, Denmark, 1953 (Höhnk); Bremerhaven, Germany, 1953 (Höhnk).

C. CAMBRENSIS Wilson, Brit. Myc. Soc. Trans. **37**: 276, f. 12–23. 1954.

Hab. Alder and Douglas fir, Aberystwyth, Wales, 1950.

C. HAMATA Höhnk, Veröff. Inst. Meeresforsch., Bremerhaven **3**(2): 211, pl. 3, f. 1–5. 1955.

Hab. Wood, Bremerhaven, Germany, 1953.

The sizes of ascospores and appendages of these species of *Ceriosporopsis* are as follows:

	Ascospores	Appendages
C. halima	23–25 × 7.8–8.5μ	23–29μ long
C. barbata	18.9–24.8 × 8.7–12.2μ	21.6μ long × 5.4–8.1μ wide at base
C. cambrensis	29–31.5 × 10.5–14.5μ	36μ long × 3.6μ wide at base
C. hamata	24.3–29.7 × 8.1–8.8μ	8–10μ long × 1.35μ wide at base

Variability in the perithecium has been observed in all of these fungi. This morphological characteristic necessitates using the ascospore itself as a primary criterion for the delimitation of the species in this genus. However, an examination of the above table indicates that intergradations in sizes do occur between the spores of the different species. The practice of establishing such new species in a genus where the extremes in ascospore dimensions of present species are not sharply defined is questionable. This is especially true in scattered collections from wood where it is difficult to determine the ranges of microdimensions and characteristics of a species. The definitive approach to this problem would be a comparison of the features of all the species of *Ceriosporopsis* in pure culture on similar substrates in the laboratory. However, this is not possible at present, and only *C. halima* has been studied extensively in culture.

In view of these observations, three species of *Ceriosporopsis*—*C. halima*, *C. cambrensis* and *C. hamata*—are considered to be autonomous species. However, *C. barbata* is reduced to synonymy with *C. halima*. The ascospores of *C. barbata* and *C. halima* are nearly alike in length and width, and have almost identical appendage characteristics. Furthermore, I have found variations in the perithecia of *C. halima* identical to those illustrated by Höhnk for *C. barbata*. The species *C. hamata* has small narrow appendages, different in appearance from those of *C. halima* or *C. cambrensis*. In the latter species, the length, and especially the width, of the ascospores is greater than that described for either *C. halima* or *C. hamata*.

Extensive studies of *C. halima* in different culture media and on wood substrates have shown the extreme morphological variability of the perithecium. Among these variable features are size and shape of the venter, length of neck, and number of necks on individual perithecia. Ascospores from oblong-cylindrical perithecia occurring on wood produce globose perithecia in agar culture. Various shapes and sizes of perithecia occurred on balsa wood sections in liquid media inoculated with spores from uniform perithecia with globose venters. Wilson observed the effect of the wood on the size and shape of the perithecia of this fungus. In alder, the perithecia were small and very irregular, expanding into the natural cavities of the xylem elements and being constricted by the walls. In softer coniferous woods, the perithecia were larger and more ovoid in shape. Also, the perithecia were less constricted in height when formed in spring wood than when formed in autumn wood.

The length of the neck of the perithecium varies noticeably among fruiting structures, both in agar, and especially among perithecia occurring at different depths within the wood. However, perithecia with long necks as well as those with no apparent neck present occurred in the same wood area both near the surface and deep within the wood itself.

Another conspicuous variable feature is the occurrence of many necks on a single perithecium. Some ascocarps have 20–30 or more necks present, with branching and irregularity noticeable in the developing necks. This production of multi-necked perithecia occurred regularly in over several hundred cultures examined, varying among cultures in the number and arrangement of necks. Such diversity in the appearance of the neck occurred on different media and wood substrates, under various physiological conditions of temperature, pH and salinity, and with different inoculum sources. Single-spore cultures derived from single-necked perithecia produced multi-necked as well as single-necked structures. Mixtures of different perithecia with diverse numbers of necks occurred regularly in individual cultures. However, some cultures had predominantly single- or bi-necked perithecia, while in others most of the perithecia had as many as 20 necks. On the wood substrate and within agar cultures, the necks of the perithecia developed on all axes, with no bending of the neck in any characteristic tropic response.

Linder reported that the imperfect stage of *C. halima* was *Helicoma salinum*. However, *H. salinum* was not observed during extensive cultural studies of *C. halima*. The only conidial stage noted was that in which branching chains of light-brown, swollen, chlamydospore-like cells occurred within the agar medium of the different cultures.

The developmental stages of the ascocarp of *C. cambrensis* were described by Wilson from material isolated from Douglas fir and alder. She reported the presence of interthecial stromatic elements or pseudoparaphyses among the asci, thus characterizing the ascocarp as a pseudoperithecium. However, the present investigator, using perithecia derived from single-spore cultures of *C. halima*, has not been able to find such pseudoparaphyses. The sections of perithecia of the fungus examined indicate that this structure is a true perithecium and not a pseudoperithecium as described by Wilson for *C. cambrensis*.

*REMISPORA Linder

Type: *R. maritima* Linder, monotype.

The perithecia of this genus are globose to subglobose, light or cream-colored, with a neck varying from truncate-conoid and eccentric to long and exserted. Paraphyses are absent. Ascospores are ovoid, ellipsoid or elongate-ellipsoid, 2-celled, hyaline, with hyaline, broad, bifurcate, tapering mustache-shaped appendage at each end. The appendages may later deliquesce or drop off.

Linder observes that the appendages of the ascospores of this genus appear to be remnants of ascus protoplasm that has differentiated during formation of the spore, rather than secondary formations originating from the spore body.

R. MARITIMA Linder, Farlowia **1**: 410, pl. 3, f. 5–9. 1944.
R. lobata Höhnk, Veröff. Inst. Meeresforsch., Bremerhaven **3**(2): 206, pl. 1, f. 1–7. 1955.

Hab. Wood, Thomaston, Maine, 1942 (Barghoorn 22b); Provincetown, Massachusetts, 1942 (Barghoorn 25, Type); Beaufort, North Carolina, 1955 (Johnson); Aberystwyth, Wales, 1951 (Wilson); Bremerhaven, Germany, 1952, 1953, 1954 (Höhnk).

In his original description of the genus, Linder noted that the neck of the perithecium was truncate-conoid and eccentric or nearly lateral. However, Höhnk in his description of *R. lobata* found the perithecia to have long-exserted necks, at times branched, with occasionally two necks occurring on individual perithecia. Thus, in the genus *Remispora* as well as in several other genera mentioned here, extreme variability in the structure of the perithecium is not uncommon. Höhnk uses this difference in perithecial shape and neck arrangement as a main criterion to establish the species *R. lobata*. However, in view of the wide range of dissimilarity in the perithecia within a single species, as observed in

species of *Lulworthia* and *Ceriosporopsis*, this characteristic is of questionable value in determining species differentiation in this group of fungi.

The ascospores of *R. maritima* and *R. lobata* have a similar range in size, and further similarity is apparent from the illustrations of the spores of the two species. The spores of *R. maritima* are (17.5–)21.5–27(–31.5) × (8–)10–11.5(–12) μ, while those of *R. lobata* are 16–21.6(–25.7) × 9.4–12.1 μ. In view of this, the latter species is relegated to synonymy with *R. maritima*.

*Antennospora gen. nov.

Perithecia aliquatenus vel omnino immersa, atra, carbonacea, globosa vel subglobosa, ostiolata, collo longit. variabili, substrato superante. Paraphyses nulli. Asci octospori, elongato-clavati, mox deliquescentes. Ascosporae ellipsoideae vel ellipsoideo-cylindraceae, bicellulares, hyalinae, utrinque appendiculis binis acuminatis terminatae.

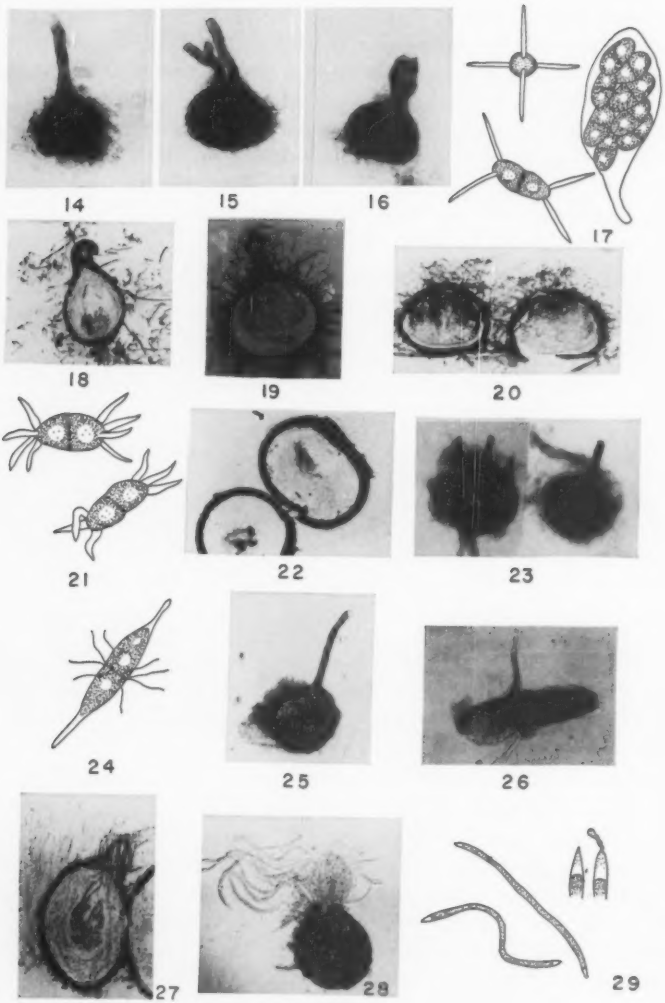
Perithecia partly to completely imbedded in substrate, becoming exposed through the erosion of the matrix, black, carbonaceous, globose to subglobose, ostiolate; neck dark, variable in length, exserted beyond substrate. Paraphyses absent. Asci 8-spored, elongate-clavate, early deliquescing. Ascospores ellipsoid to ellipsoid-cylindric, 2-celled, hyaline, with two hyaline acuminate appendages at each end.

Type: *A. caribbea* Meyers.

This genus is characterized by 2-celled ascospores with 2 acuminate appendages at each end of the spore. The arrangement of these appendages is diagnostic of the genus in that they present a cruciform appearance when the spore is viewed terminally.

Morphological variation in *Antennospora* appears in the size of the mature perithecium, and in the size, shape and number of necks, as well as in the insertion of the neck on the venter. These variations have been noted following the examination of wood from many areas, including wood cultures in laboratory tanks, involving thousands of mature perithecia. Thus, it has been possible to determine the type of ascocarp which is more representative of the species as well as the extent and nature of its variability. Perithecial variability in *Antennospora* is not as striking as is apparent in the perithecia of *Ceriosporopsis* and *Lulworthia*.

Bulbous swellings on the neck wall may occur, with occasionally a general distortion of the neck itself. This often results in short irregular necks, greatly enlarged in diameter. Further malformations have also been observed. Single ascocarps with two distinct elongate necks are



FIGS. 14–29.

FIGS. 14–18. *Antennospora caribbea*. 14. Single-necked perithecium, × 43. 15. Perithecium with two necks, × 59. 16. Perithecium with short malformed neck, × 40. 17. Ascus, × 190, and ascospores, × 218. Spore on top shows typical cruciform appearance when viewed terminally. 18. Longitudinal section of young perithecium, × 80. FIGS. 19–21. *Arenariomyces salina*. 19. Longitudinal section

found occasionally, as are compound ascocarps with two necks resulting from the fusion of venters. Infrequently, perithecia occur with a slightly elongate venter and an eccentric neck. However, the majority of ascocarps of this genus have a globose venter with a central neck.

The position of the fruiting structure in the wood tissue varies from completely imbedded perithecia with long exserted necks to partly or mostly exposed ones with necks of variable length. In the imbedded perithecia, extreme modification of the venter into an elongate-cylindrical or irregular structure, conforming to the shape of the surrounding wood element, has not been observed. Undoubtedly, the erosion of the wood tissue surrounding the ascocarp during stages of development affects the final relationship of the fruiting structure to the substrate.

A. caribbea sp. nov.

FIGS. 14–18

Perithecia aliquatenus vel omnino immersa, atra, carbonacea, globosa vel subglobosa, ostiolata, 371–514 × 249–285 μ, collo apicali, 142–185 × 36–42 μ, praedita. Asci octospori, elongato-clavati. Ascosporae ellipsoideae vel elongato-ellipsoideae, 27–32 × 9–10 μ, bicellulares, hyalinae, haud ad septum constrictae, cellula quaque vacuolo uno magno praedita, appendiculatae; appendiculae acuminatae, 22–28 μ long., ad basim 1.2–1.5 μ diam., in paribus terminalibus decussatis instructae.

Perithecia partly to completely imbedded in substrate, black, carbonaceous, globose to subglobose, ostiolate, variable in size, 371–514 × 249–285 μ, with dark fuscous broad, branching hyphae arising from base of venter; neck apical, 142–185 × 36–42 μ, dark, becoming hyaline distally, occasionally swollen and irregular. Asci 8-spored, elongate-clavate, deliquescent at maturity. Ascospores ellipsoid or elongate-ellipsoid, 27–32 × 9–10 μ, 2-celled, hyaline, no constriction at the septum, each cell with a single large vacuole, 7–8 μ in diameter; appendages hyaline, acuminate, 22–28 μ long and 1.2–1.5 μ wide at base, two at each end, inserted subterminally, the pair at one end arranged in a plane at right angles to that of the pair at the opposite end, presenting a cruciform appearance when viewed terminally.

---

of young ostiolate perithecium, × 77. 20. Longitudinal section of young non-necked perithecia, × 77. 21. Ascospores, × 367. FIGS. 22–24. *Peritrichospora integra*. 22. Longitudinal section of young, non-necked, thick-walled perithecium, × 87. 23. Ostiolate perithecia. Perithecium on left has three distinct necks, × 27, × 29. 24. Ascospore, × 590. FIGS. 25–29. *Lulworthia floridana*. 25. Ostiolate perithecium with globose venter, × 53. 26. Elongated perithecium imbedded in wood, × 60. 27. Longitudinal section of young perithecium, × 67. 28. Globose non-necked perithecium with exudation of ascospores, × 70. 29. Ascospores, × 87. Insert shows appendages, × 500. One at right has exudation of cytoplasmic material from apex.

Hab. Wood, including yellow pine and northern spruce, Biscayne Bay, Florida, 1952–1954; Cedar Key, Florida, Dec., 1953; Key West, Florida, Nov., 1953; Port Aransas, Texas, Feb., 1954; Carmen, Mexico, May, 1954; Guantamano Bay, Cuba, May, 1954; Port-au-Prince, Haiti, May, 1954; San Juan, Puerto Rico, Apr., 1954; Antigua, B.W.I., Feb., 1954; Martinique, F.W.I., Apr., 1954; Trinidad, B.W.I., Mar., 1954; Coco Solo, Canal Zone, Dec., 1953; Belize, British Honduras, May, 1954; Bimini, The Bahamas, Nov., 1953; Exuma, The Bahamas, June, 1954; Eleuthera, The Bahamas, Mar., 1954; Abaco, The Bahamas, May, 1954; Papeete, Tahiti, 1956.

A. caribbea is the Form No. 2 referred to previously (Meyers, **1953**). Longitudinal sections of young perithecia of *A. caribbea* show that the ascocarp develops in the manner characteristic of a true perithecium. No paraphyses or pseudoparaphyses are present.

The different geographic isolates of the fungus mentioned above appear similar in spore characteristics and in the range in size and shape of the ascocarp.

Collection of *A. caribbea* from subtropical and tropical areas, together with its absence from more temperate marine environments, suggests that it is primarily an inhabitant of warmer oceanic areas. Collections of wood in New England and Beaufort, North Carolina, as well as in scattered collections along the Atlantic Seaboard above the Florida peninsula, have failed to yield this fungus. In the areas sampled, the species appears to be a regular and abundant inhabitant of the marine microfloral population.

*ARENARIOMYCES Höhnk

Palomyces Höhnk, Veröff. Inst. Meeresforsch., Bremerhaven **3**(2) : 212. 1955.

Type: *A. trifurcatus* Höhnk.

The perithecia of this genus are spherical to globose to elongate, dark fuscous to black, carbonaceous to sub-carbonaceous and ostiolate. The neck varies from a small papillate type to one long and cylindrical. Paraphyses are absent. The asci are 8-spored, elongate-clavate, deliquescing early. Ascospores are ovoid to fusiform, 2-celled, hyaline and appendaged at both ends.

A. TRIFURCATUS Höhnk, Veröff. Inst. Meeresforsch., Bremerhaven **3**(1) : 30, pl. 2, f. 1–6. 1954.

Hab. Sand particles, Bremerhaven, Germany, 1952–1953.

A. quadri-remis (Höhnk) comb. nov.

Palomyces quadri-remis Höhnk, Veröff. Inst. Meeresforsch., Bremerhaven **3**(2): 13, pl. 4, f. 1–3. 1955.

Hab. Copenhagen, Denmark, 1953.

A. salina sp. nov.

FIGS. 19–21

Perithecia immersa vel semisuperficialia, per lignum detritum denudata, globosa (266–308 × 336–392 μ) vel elongata (300–425 × 210–280 μ), atrofusca, membranacea vel paulo carbonacea, interdum striata, ostiolata, collo apicali, papillato, vel mera ventris apertione, vel elongato, cylindraceo. Asci octospori, elongato-clavati, mox deliquescentes. Ascosporae ovoideae vel ellipsoideae, apicibus rotundatis, bicellulares, 19–23 × 8.1–11 μ, hyalinae, quaque cellula vacuolum magnum continente, appendiculatae, appendiculis acuminatis, curvatis, saepe unguiformibus, trinis vel quaternis e finibus ambobus posteriore loco orientibus, 12–16 μ long., ad basim 1.5–2.3 μ diam.

Perithecia immersed to partly superficial, becoming exposed by erosion of the wood, globose, 266–308 × 336–392 μ, to elongate, 300–425 × 210–280 μ, dark fuscous, membranaceous to slightly carbonaceous, occasionally ridged, ostiolate; neck apical and papillate, or merely an opening in the venter, to elongate and cylindrical. Asci 8-spored, elongate-clavate, early deliquescing. Ascospores ovoid to ellipsoid with rounded apices, 2-celled, 19–23 × 8.1–11 μ, hyaline, each cell with a large vacuole, appendaged; appendages acuminate, curved, often claw-like, 3–4 at each end, arising posteriorly, 12–16 μ long and 1.5–2.3 μ wide at the base.

Hab. Wood, including yellow pine, Biscayne Bay, Florida, 1953–1954; Bimini, The Bahamas, Nov., 1953; Belize, British Honduras, May, 1954.

A. salina is the form No. 7 reported previously (Meyers, **1954**). Sectioned material of *A. salina* has shown the absence of pseudoparaphyses or paraphyses in the developing perithecium. A thick brown hyphal mat commonly occurs over the surface of the perithecia developing on the wood substrate. In some instances, perithecia are clumped together on the wood with their venters variously merged.

The sizes of the ascospores and appendages of these species are as follows:

	Ascospores		Appendages	
	Shape	Size	Number at each end	Length
A. trifurcatus	fusiform	28.3–32.5 × 9.3–10.8μ	3	24.3–32.5μ
A. quadri-remis	elliptical	21.6–29.7 × 8.1–10.8μ	4	14.8–20μ
A. salina	ovoid to elliptical	19–23 × 8.1–11μ	3–4	12–16μ

Höhnk described the perithecia of the genus *Arenariomyces* as being typically globose or subglobose, black, carbonaceous, and rarely with a small central papillate neck. In the species *A. trifurcatus*, the spores are liberated through a break in the wall of the perithecium. In a subsequent paper, Höhnk describes *Palomyces*, a genus characterized by globose to subglobose, membranous, light-colored perithecia with long cylindrical necks. Occasionally one or more necks occur on individual perithecia.

Meyers (**1954**) described Form No. 7 (*A. salina*), a fungus with appendaged ascospores similar to those of *Arenariomyces* and *Palomyces*. Form No. 7 has subglobose to elongate, membranous to subcarbonaceous perithecia with a neck varying from extremely small and papillate to long and cylindrical. The earlier studies of Form No. 7, using wood exposed in Biscayne Bay, Florida, did not reveal perithecia with distinct necks. The majority of perithecia examined were without necks or cleistothecium-like, with the spores liberated through an opening or break in the wall of the perithecium. However, subsequent examination of balsa wood sections infected in laboratory tanks of aerated sea water by pure cultures of *A. salina* have shown an intergradation of perithecial types. These vary from non-necked to long-necked structures, with both globose and slightly elongate types of perithecia present.

The ascospores of *Arenariomyces, Palomyces* and Form No. 7 are fusiform, elliptical or ovoid, 2-celled, hyaline and appendaged at both ends. In all three fungi the ascospores have a single large vacuole and occasional smaller ones in each cell. The relative length-width ratio of the ascospores is similar in these genera, as is also the width of the ascospore itself. The difference in number of appendages on the ascospore in these fungi does not appear significant enough to warrant generic distinction, especially since Form No. 7 has three as well as four appendages present. Of greater significance is the fact that in all three fungi the arrangement and general characteristics of the appendages are identical. Thus, from the standpoint of the ascospore itself, *Arenariomyces, Palomyces* and Form No. 7 can be placed within a single genus. However, the differences in spore and appendage length between these fungi suggests that they are separate species.

In view of the above similarities in the type of ascospore and the variation in the structure of the perithecium within a single genus, *Arenariomyces, Palomyces* and Form No. 7 can be considered synonymous. Since the genus *Arenariomyces* Höhnk has priority, *Palomyces* is relegated to synonymy.

*HALOSPHAERIA Linder

Type: *H. appendiculata* Linder, monotype.

The perithecia are ovoid, dark fuscous or nearly black above, subhyaline below, ostiolate. The neck is eccentric and oblique, black, stout and short-cylindric. Paraphyses are absent. Asci 4-spored, broadly clavate. Ascospores are ellipsoid, hyaline, 1-septate, with (2–3–)4 slender hyaline appendages of which two are usually at the septum and one at each end.

H. APPENDICULATA Linder, Farlowia **1**: 412, pl. 4, f. 4–7. 1944.

Hab. Wood, Newburyport, Massachusetts, 1942 (Barghoorn 19c, Type); Provincetown, Massachusetts, July, 1955 (Meyers).

A collection of *H. appendiculata* which I examined showed ascospores that were regularly 4-appendaged, with two appendages at the septum and one at each end. The spores described by Linder for the species with less than four appendages probably had lost one or more appendages through deliquescence or by dropping off.

*PERITRICHOSPORA Linder

Type: *P. integra* Linder, lectotype (S. P. M.).

The perithecia of this genus are globose, black with occasionally a metallic luster, subcarbonaceous to carbonaceous and ostiolate. The neck varies from small and papillate to long and exserted with several necks on individual perithecia. Paraphyses are absent. The asci are 8-spored, broadly fusoid to elongate, early deliquescent. Ascospores are fusoid, occasionally slightly curved, 1–5-septate, hyaline, with a single appendage at each end and cilium-like processes around the central septum.

The genus *Peritrichospora* is separated from the other marine pyrenomycetous genera with appendaged spores by the numerous cilium-like processes around the central septum and by the carbonaceous spherical perithecia, usually with small ostioles.

P. LACERA Linder, Farlowia **1**: 415, pl. 5, f. 1–5. 1944.

Hab. Wood, Provincetown, Massachusetts, 1942 (Barghoorn 28b, Type).

P. INTEGRA Linder, Farlowia **1**: 414, pl. 5, f. 6–9. 1944.
Arenariomyces cinctus Höhnk, Veröff. Inst. Meeresforsch., Bremerhaven **3**(1): 28, pl. 1, f. 1–5. 1954.

FIGS. 22–24

Hab. Wood, including pine, Woods Hole, Massachusetts, 1942 (Barghoorn 10, Type); Provincetown, Massachusetts, July, 1955 (Meyers); Gloucester Point, Virginia, July, 1955 (Meyers); Beaufort, North Carolina, August, 1955 (Johnson); Biscayne Bay, Florida, 1953–1954 (Meyers); Aberystwyth, Wales, 1951 (Wilson); sand particles, Bremerhaven, Germany, 1952–1953 (Höhnk).

Linder described the perithecia of this genus as being globose and carbonaceous with a very small papillate central ostiole. In my studies of *P. integra,* similar perithecia have been observed as well as perithecia with long necks and more than a single neck on an individual perithecium. These latter long-necked perithecia are subcarbonaceous to membranous rather than hard and brittle as are those of the non-necked type. The value of these various perithecial characteristics as accurate criteria in this genus is questionable.

Höhnk (**1954**) described the genus *Arenariomyces,* a genus with two species, *A. cinctus* and *A. trifurcatus.* The latter species has been discussed under the genus *Arenariomyces,* which includes 2-celled ascospores with 3–4 appendages at each end. However, comparison of Höhnk's figures of *A. cinctus* with those of *P. integra* definitely establishes that these two species are synonymous. Since the latter species has priority, *A. cinctus* Höhnk is reduced to synonymy.

The ascospores of *P. integra* are 1-septate, while those of *P. lacera* are 5-septate. Linder mentions the occurrence of pseudosepta, or annular thickenings deposited by the cytoplasm, in the spores of *P. integra.* However, examination of the spores of this species has failed to reveal such pseudosepta. The ascospores of *P. integra,* exclusive of appendages, are 21.5–33 × 6.5–8.3 μ, while those of *P. lacera* are 41–53(–62.8) × 11.5–15 μ. The examination of perithecial sections of *P. integra* has shown the absence of pseudoparaphyses, with the perithecium developing in the manner of a true perithecium.

P. integra has been collected from Biscayne Bay, Florida, on calcareous deposits over the wood surface, as well as on the wood itself. The perithecia occurring on the calcareous material have a hard black flattened base, tenaciously adhering to the substrate surface. The same structural condition was observed in cultures of the fungus, in which vigorous perithecial production occurred on the side of the glass dish.

*LULWORTHIA Sutherland

Halophiobolus Linder, Farlowia **1**: 415. 1944.
Type: *L. fucicola* Suth.

Perithecia superficial or immersed, globose to elongate-ellipsoid, membranous to subcarbonaceous, fuscous to black, ostiolate, with or without necks, the latter papillate to elongate and cylindrical, variable in length, occasionally irregular and branched, with often more than one neck on individual perithecia. Paraphyses are absent. Asci elongate-clavate, coiled around within the venter, 8-spored, deliquescent when mature. Ascospores filamentous, hyaline, straight to sigmoid or variously curved, generally non-septate, provided at each end with a conical hyaline appendage.

The genus *Lulworthia* was described first by Sutherland in 1915 on the brown alga *Fucus vesiculosus* in the British Isles. Sutherland compared *Lulworthia* with other scolecosporous genera, including *Ophiobolus,* and determined that *Lulworthia* could be separated from these genera on the basis of its appendaged and non-septate filamentous spores, together with the absence of paraphyses. In *Ophiobolus,* paraphyses are present and the ascospores fragment into unicellular portions at maturity.

Petrak (Sydowia **6**: 388. 1952) placed *Halophiobolus halima* (syn. *Lulworthia halima*), *Halophiobolus medusa* (syn. *Lulworthia medusa*) and *Halophiobolus maritimus* (syn. *Ophiobolus maritimus*) in the genus *Linocarpon* Syd. (Ann. Myc. **15**: 210. 1917). The latter genus is characterized by filamentous non-appendaged ascospores and perithecia with a pseudo-clypeus. Petrak considers the spore appendages of *Lulworthia* to be thickenings of the epispore and not "true" appendages. However, my studies of the species of *Lulworthia* indicate that the spore appendage is of cytoplasmic origin and is a distinct and regular morphological characteristic. An examination of the diagnostic features of *L. halima* and *L. medusa* shows that these two species are assigned correctly to *Lulworthia* and do not belong in *Linocarpon. Ophiobolus maritima* has non-appendaged ascospores that fragment at maturity and does not appear related to *Lulworthia.* As discussed subsequently, *O. maritima* was placed incorrectly in the genus *Halophiobolus,* and was assigned to *Linocarpon* by Petrak on this basis. A pseudo-clypeus is not present in either *Lulworthia* or *Ophiobolus.*

Cribb and Cribb (**1955**) regarded the genus *Halophiobolus* Linder as a synonym of *Lulworthia* Suth. and transferred the four species of the former genus described by Linder, together with the three species transferred to that genus by Linder from *Ophiobolus,* to the genus

Lulworthia Suth. Cribb and Cribb observed that both *Lulworthia* and *Halophiobolus* have similar diagnostic features, *i.e.*, marine environment, 8-spored asci, absence of paraphyses, and continuous filamentous ascospores with short hyaline apical appendages. Linder, in establishing the genus *Halophiobolus*, appears to have overlooked the paper by Sutherland in which he described *Lulworthia*. This latter paper is not listed by Linder in his list of references, although the four other papers by Sutherland on marine fungi are cited.

Considerable variability in the character of the perithecium within the various species of *Lulworthia* has been observed both on wood and in artificial culture. A discussion of this variability is presented below.

Cultural studies upon wood and in agar have indicated that the characteristics of the ascospore, such as size and appendage length, are more reliable features than are those of the perithecium itself. The ascospores of 12 different geographic isolates of *Lulworthia* have been studied in our laboratory over a period of several years. Measurements of spore length and width and appendage length have been made within individual isolates in liquid culture as well as on various agar media. This has permitted a comparison of spore sizes between isolates in addition to allowing a careful analysis of the range of spore dimensions within each isolate. Within any single species of *Lulworthia* as described here, ascospore length varies over a fairly definite range. These dimensions follow those of a typical population curve. For different isolates of a species, the range of the peak of the curve on either side of the midpoint is narrow. This narrow range or distributional peak is fairly constant for the individual species. Even when two such species overlap slightly in the size range, the distributional peak is distinctly different for the 2 species. Thus, the differentiation of species insofar as ascospore length is concerned can be tabulated by a comparison of the distributional or population peaks of the individual species.

Mature ascospores have been used in all of these studies and have been measured in sea water as well as in picric acid-aniline blue and in cotton blue-lactophenol stain. The appendages are apparent especially in the latter two materials.

Morphological variability in *Lulworthia*

Length of neck. The length of the neck of mature perithecia varies considerably, even on perithecia produced in single-spore cultures. Growth of the neck occurs well after the venter and ascospores have matured fully. Within a single species, perithecia may vary from

structures lacking necks to those with long straight or curved necks. Perithecia with more than a single neck are of common occurrence in agar and on wood. These perithecia may have from two to many individual necks, or a single neck may branch repeatedly. Malformation of the neck tissue is not uncommon.

Color and shape of the venter. Various gradations in color from hyaline to nearly black have been found in perithecia produced in single-spore cultures. Mature spores have been found in light-colored perithecia, which later become fuscous to black. In these instances, the color attributed to the perithecium may depend upon the stage of development when it was collected and described, or to environmental factors. The nature of the substrate seems to have an influence on the amount of melanin produced within the perithecium.

Size and especially shape of the perithecium are further variable morphological characteristics. While the general shape of the perithecia of a species grown on a homogeneous medium such as agar is comparatively uniform, this is not so with perithecia produced on a heterogeneous substrate such as wood. Studies of pure cultures of species of *Lulworthia* on wood have shown noticeable dissimilarities in shape of the perithecia, from globose to elongate, with apical or eccentric necks. The shape of the ascocarp is affected by the type of surrounding wood tissue, so that perithecia developing within wood vessels and tracheids often become elongate in appearance.

These observations suggest that differences among random samples obtained on wood submerged in sea water may represent normal perithecial variation rather than species characteristics.

Relation of perithecia to substrate. On a natural substrate, such as wood submerged in sea water, it is not possible to consider position of the ascocarp on or in the wood as a primary diagnostic feature. The erosion of the outer wood tissues during prolonged exposure in sea water, in addition to removing superficial perithecia, may expose imbedded perithecia or even cause the destruction of exserted necks. Diverse types of wood, varying in anatomical structure and resistance to deterioration, may exhibit differences in relationship of the perithecia to the wood. Similarly, the latter association is affected by the area of initial infection, whether it is directly through the outer wood surface, through the ends of the vessels or through ray cells. In a single species, perithecia may occur normally at varying depths within the wood, with some perithecia even present on the surface of the wood.

In the following key, the species of *Lulworthia* have been separated on the basis of differences in ascospore dimensions.

KEY TO THE SPECIES OF LULWORTHIA

1. Ascospores less than 100 μ long ..... *L. fucicola*
1. Ascospores more than 100 μ long 2
2. Spores between 100 μ and 500 μ long 3
2. Spores over 500 μ long ..... *L. grandispora*
3. Spores 150–200 μ long ..... *L. salina*
3. Spores more than 200 μ long 4
4. Spores 230–320 μ long 5
4. Spores 320–425 μ long 6
5. Hyaline appendages 3–4 μ long ..... *L. halima*
5. Hyaline appendages 7–8–14 μ long ..... *L. floridana*
6. Hyaline appendages 3.6–4.4–7 μ long ..... *L. medusa* var. *biscaynia*
6. Hyaline appendages 7–8.4 μ long ..... *L. medusa*

L. FUCICOLA Sutherland, Brit. Myc. Soc. Trans. **5**: 259, pl. 5, f. 4–7. 1916.

Halophiobolus cylindricus Linder, Farlowia **1**: 416, pl. 6, f. 12–14. 1944.

L. cylindrica (Linder) Cribb and Cribb, Papers, Univ. Queensland Dept. Bot. **3**(10): 79. 1955.

Hab. *Fucus vesiculosus,* Dorset Coast, British Isles (Sutherland); wood, Provincetown, Massachusetts, 1942 (Barghoorn 23); July, 1955 (Meyers).

This species, according to Sutherland, has a globose perithecium with a papillate ostiole or none at all. Linder described *Halophiobolus cylindricus* as having a cylindrical or sub-cylindrical perithecium with a long projecting neck arising from a bulbous pseudoparenchymatous base. Cribb and Cribb use these differences in structure of the perithecium to separate *L. fucicola* from *L. cylindrica.* The ascospores of the two are very similar in size. Those of *L. fucicola* are 70–100 × 4.5–5.5 μ while those of *L. cylindrica* are 74–82.5 × 5 μ.

The writer's examination of the perithecia of species of *Lulworthia* on wood and in culture has shown that differences in perithecial characteristics among isolates can hardly be used as a primary criterion in defining species of this genus. The writer was able to isolate and culture specimens of *L. cylindrica* from collections obtained in the type locality, the Provincetown, Massachusetts, area. The perithecia in culture were globose with a central exserted neck, quite unlike those ascribed to this species by Linder. The original perithecia on the wood from which the ascospore inoculum was taken were cylindrical in shape and came within the limits of Linder's description. On the basis of these obser-

vations, perithecial characteristics alone cannot be used to separate *L. cylindrica* from *L. fucicola.*

The spore and appendage dimensions of the globose-type perithecia in culture are nearly identical with the dimensions in *L. cylindrica* as given by Linder. Unfortunately, Sutherland does not give the length of the appendage in *L. fucicola.* However, on the basis of similarity in spore size in *L. fucicola* and *L. cylindrica,* the latter is reduced to synonymy with *L. fucicola.*

L. grandispora sp. nov.

Perithecia superficialia vel immersa, globosa vel pyriformia, atrofusca vel atra, ventri 180–230 × 180–296 μ, collo 75–1400 μ long., 15–33 μ lat., recto vel curvulo, irregulari. Asci elongato-cylindracei, octospori. Ascosporae filiformes, aseptatae, 565–647–756 × 2.9–3.5–4.3 μ, hyalinae, utrinque appendicula attenuata hyalina 3.6–3.7–4.3 μ long. terminatae.

Perithecia superficial or immersed, globose to pyriform, dark fuscous to black, venter 180–302 × 180–296 μ, with outgrowths of dark hyphae around venter; neck long, variable in length, 75–1400 × 15–33 μ, straight to curved and irregular, with two necks occasionally occurring on a perithecium. Paraphyses absent. Asci long-cylindrical, deliquescing at maturity, leaving spores in bundles of eight. Ascospores filiform, non-septate, 565–647–756 × 2.9–3.5–4.3 μ, hyaline, straight or variously curved, with a hyaline conoid appendage, 3.6–3.7–4.3 μ long, at each end.

Hab. Yellow pine wood, Cedar Key, Florida, Dec., 1953.

This species clearly is distinguished from the other species of *Lulworthia* by the extremely long ascospores. The asci and spores of *L. grandispora,* as well as those of the other species of *Lulworthia* described here, characteristically are curled within the venter. This bending or curling of the ascospores as they mature within the perithecium is apparent especially in *L. grandispora* where the spores are often 2 to 3 times as long as the diameter of the ascocarp. Immediately following the release of the ascospores of this genus from the ostiole of the perithecium, the spores commonly assume a "C" or "S" configuration, suggesting previous curling of the spores during their developmental stages within the venter.

A further distinctive feature of *L. grandispora* is the continuous elongation of the perithecial necks in culture. Even in perithecia with necks over 1400 μ long and often irregularly curved, it is possible to observe the passage of mature spores through the neck canal.

L. SALINA (Linder) Cribb and Cribb, Papers, Univ. Queensland Dept. Bot. **3**(10) : 80. 1955.

Halophiobolus salinus Linder, Farlowia **1** : 419, pl. 6, f. 8–11. 1944.

Halophiobolus opacus Linder, Farlowia **1** : 417, pl. 6, f. 1–5. 1944.

Halophiobolus longirostris Linder, Farlowia **1** : 418, pl. 6, f. 6–7. 1944.

Lulworthia opaca (Linder) Cribb and Cribb, Papers, Univ. Queensland Dept. Bot. **3**(10) : 79. 1955.

Lulworthia longirostris (Linder) Cribb and Cribb, Papers, Univ. Queensland Dept. Bot. **3**(10) : 80. 1955.

Hab. Wood, Portsmouth, New Hampshire, 1942 (Barghoorn 1d, 11) ; Fall River, Massachusetts, 1942 (Barghoorn 4) ; Provincetown, Massachusetts, 1942 (Barghoorn 14, 26) ; Woods Hole, Massachusetts, 1942 (Barghoorn 20) ; Saybrook, Connecticut, 1942 (Barghoorn 17) ; Maine, 1942 (Barghoorn 22) ; Bremerhaven, Germany, 1953 (Höhnk) ; pine panels, Beaufort, North Carolina, 1955 (Johnson).

L. salina, L. opaca and *L. longirostris* are separated by Linder on the basis of differences in perithecial characteristics, *e.g.*, size and shape of the perithecium and color and thickness of the wall of the perithecium. In all three descriptions, the size of the ascospore and length of the appendage is nearly identical. In *L. salina,* the spores are 180–200 × 3.3 μ, in *L. opaca,* 155–190 × 3.3–4 μ, and in *L. longirostris,* 155–200 × 3.3–4 μ. The length of the appendage is 7.5–8.5 μ in all three species.

While variations in color, shape and size of the perithecium, and in length of the neck do exist among the three species, such differences, in view of previous discussion, are not sufficient for the separation of species in this genus. In the light of this variability in the perithecia of *Lulworthia,* it would seem illogical to maintain three separate species almost solely on the basis of the shape and color of the perithecium as well as on the cells comprising the perithecial wall. Since ascospore characteristics are believed to represent a more reliable basis for specificity, the three species are considered synonymous.

L. HALIMA (Diehl & Mounce) Cribb & Cribb, Papers, Univ. Queensland Dept. Bot. **3**(10) : 80. 1955.

Ophiobolus halimus Diehl & Mounce, Canad. Jour. Res. **11** : 245–246, f. 1, 3–9. 1934.

Halophiobolus halimus (Diehl & Mounce) Linder, Farlowia **1** : 419. 1944.

Linocarpon halima (Diehl & Mounce) Petr., Sydowia **6** : 388. 1952.

Hab. *Zoster marina,* St. Andrews, New Brunswick, 1933.

The perithecia of *L. halima* are spherical, black, 240–435 μ in diam-

eter, with an acute conoid rostrum, up to 278 μ in length and 260 μ in basal diameter. Occasionally more than one neck occurs on a perithecium. This type of perithecium is different from that of the following species, *L. floridana*, in which the perithecium has a long slender neck not exceeding 50 μ in diameter over its entire length.

Mounce and Diehl described the ascospores of *L. halima* as being 260–308 × 2–4 μ, with hyaline appendages 3 μ long. Examination of the type material of this species showed similar dimensions, except for the occurrence of spores as small as 230 μ long, and appendages as long as 4.2 μ.

L. floridana sp. nov.

FIGS. 25–29

Perithecia superficialia vel immersa, globosa vel pyriformia, ventri 187–216 × 173–216 μ, atro, collo interdum papillato, plerumque elongato, 280–720 × 28–30 μ, recto vel irregulari, curvulo, paraphysibus nullis. Asci elongato-cylindracei, maturi deliquescentes. Ascosporae filiformes, aseptatae, 230–266–320 × 3.6–4.2–5.7 μ, hyalinae, rectae vel curvulae, utrinque appendicula hyalina 7–8–14 μ long. ornatae.

Perithecia superficial or immersed, globose to pyriform, venter 187–216 × 173–216 μ, black; neck occasionally papillate, mostly elongate, 280–720 × 28–30 μ, straight to irregular and curved, dark, becoming hyaline at apex, with two or three necks, occasionally branched, commonly occurring on a perithecium. Paraphyses absent. Asci long-cylindrical, deliquescing at maturity, leaving spores in bundles of eight. Ascospores filiform, non-septate, 230–266–320 × 3.6–4.2–5.7 μ, hyaline, straight or variously curved, with a hyaline conoid appendage, 7–8–14 μ long, at each end.

Hab. Wood, including yellow pine, Biscayne Bay, Florida, 1952–1954; Grand Cayman, Cayman Islands, Apr., 1954; Martinique, French West Indies, Apr., 1954; Guantanamo Bay, Cuba, May, 1954; Provincetown, Massachusetts, July, 1955.

L. floridana and *L. halima* have ascospores of about the same length. The spores of the former species are 230–320 μ long, while those of the latter species are 260–308 μ in length. However, in *L. floridana* the appendages are 7–14 μ long, while in *L. halima* they are 3–4.2 μ long. Examination in culture and on wood of the five different geographic isolates of *L. floridana* has demonstrated the reliability of the size of the spore appendage as a diagnostic feature of this species. The considerable difference in length of the appendages of *L. floridana* and *L. halima* warrants separating these as two distinct species. The perithecia of *L. floridana* are characteristically long-necked, and in those perithecia

where short necks occur occasionally, the necks are never with an extremely broad base and conoid as described in *L. halima* by Diehl and Mounce.

L. MEDUSA (Ell. & Ev.) Cribb & Cribb, Papers, Univ. Queensland Dept. Bot. **3**(10) : 80. 1955.
Ophiobolus medusa Ellis & Everhart, Jour. Myc. **1** : 150. 1885.
Halophiobolus medusa (Ell. & Ev.) Linder, Farlowia **1** : 419. 1944.
Linocarpon medusa (Ell. & Ev.) Petr. Sydowia **6** : 388. 1952.
Lulworthia longispora Cribb & Cribb, Papers, Univ. Queensland Dept. Bot. **3**(10) : 80, f. 2, a–g. 1955.

Hab. *Spartina* sp., New Jersey, 1884; *Avicennia marina* var. *resinfera,* and wood and bark, Queensland, Australia, 1954, 1955 (Cribb & Cribb) ; wood and *Spartina alterniflora,* Beaufort, North Carolina, 1955 (Johnson).

Ellis and Everhart described the spores of *L. medusa* as being nearly 400 μ long × 3–3.5 μ wide, but failed to report the presence of the spore appendages. Mounce and Diehl, in an examination of the type specimen, described the occurrence of inflated, somewhat flattened, appendages, but failed to note their length. Examination of the type specimen showed the appendages to be 7–8.4 μ long. All the spores examined had straight, tapering lance-like tips, with no swelling or flattening similar to that described by Mounce and Diehl. The latter characteristic is believed due to an artifact.

Cribb and Cribb, in describing the species *L. longispora,* used the questionable character of the inflated appendage of *L. medusa* in distinguishing these two species. The ascospores of *L. longispora* are 330–450 μ long, while those of *L. medusa* are reported to be about 400 μ long. However, examination of the type material of *L. medusa* by the present investigator showed ascospores as small as 322 μ in length. The length of the appendage is quite similar in both species. In *L. longispora,* the appendages are 6–9 μ long, while those of *L. medusa* are 7–8.4 μ long. In view of these similarities, and the absence of inflated appendages in type material of *L. medusa,* it is doubtful that *L. longispora* can be maintained as a separate species. Accordingly, the latter species is relegated to synonymy.

L. MEDUSA var. **biscaynia** var. nov.

Perithecia superficialia vel immersa, globosa, atrofusca vel atra, ventri 144–288 × 144–273 μ, collo longitudine variabili, 400–792 × 13–33 μ, recto vel curvulo, para-

physibus nullis. Asci elongato-cylindracei, maturi deliquescentes. Ascosporae filiformes, aseptatae, 322–384–425 × 2.8–3.5–3.7 μ, hyalinae, rectae vel curvulae, utrinque appendicula hyalina 3.6–4.4–7 μ long. ornatae.

Perithecia superficial or immersed, globose, dark fuscous to black, venter 144–288 × 144–273 μ; neck long, variable in length, 400–792 × 13–33 μ, straight or curved, with occasionally two necks on a perithecium. Paraphyses absent. Asci long-cylindrical, deliquescing at maturity. Ascospores filiform, non-septate, 322–384–425 × 2.8–3.5–3.7 μ, hyaline, straight or curved, with a hyaline appendage, 3.6–4.4–7 μ long, at each end.

Hab. Wood, including yellow pine, Bermuda, Feb., 1954; Key West, Florida, Dec., 1953; Biscayne Bay, Florida, 1952–1954; San Juan, Puerto Rico, Apr., 1954; Abaco, Exuma, The Bahamas, June, 1954.

The length of the ascospores of this fungus falls within the range of that given for *L. medusa* by Ellis and Everhart. Similarly, the ascospores of *L. longispora* Cribb & Cribb are equal in length to the spores of this variety. However, in both *L. medusa* and *L. longispora,* the length of the spore appendage is greater than that observed for *L. medusa* var. *biscaynia.* In the latter fungus, the appendages are 3.6–7 μ long, while in *L. medusa* and *L. longispora* the appendages are 7–8.4 μ and 6–9 μ long, respectively. This difference in appendage length appears to be sufficiently large to warrant the separation of these isolates with shorter appendages into a separate taxon. However, since the difference in appendage length in this group is not as striking as that observed between *L. halima* and *L. floridana,* the former is given only varietal consideration.

*TRAILIA Sutherland

Type: *T. ascophylli* Suth., monotype.

T. ASCOPHYLLI Suth. Brit. Myc. Soc. Trans. **5**: 149, pl. 3, f. 2, 1–3. 1915.

Hab. *Ascophyllum nodosum,* Orkney Islands; Aberystwyth, Wales, 1951 (Wilson).

The perithecia of this genus are soft and white with a long narrow, straight or curved, cylindrical neck. Paraphyses are absent. The ascospores are filamentous, tapering, septate, hyaline, and bent double and coiled in the ascus. The ascospores are approximately 90–100 × 3–3.5 μ, tapering to 1 μ, non-septate at first, later developing 1–3 septa in the wide end of the spore only.

OPHIOBOLUS Reiss

O. LAMINARIAE Suth. Brit. Myc. Soc. Trans. **5**: 147, pl. 3, f. 1, 1–4. 1915.

Hab. *Laminaria digitata,* Orkney Islands.

The filamentous spores of *O. laminariae* Suth. which break into cylindrical unicellular portions at maturity are typical of the spores of *Ophiobolus* Reiss. The absence of spore appendages further separates this species from members of the genus *Lulworthia.* However, the absence of paraphyses in *O. laminariae,* together with the presence of long-necked (175–250 μ in length) perithecia, are characteristics atypical of *Ophiobolus.* In view of the filamentous fragmenting ascospores, similar to those present in *O. maritimus, O. laminariae* is retained in *Ophiobolus.* Sutherland himself questions the systematic position of *O. laminariae* in his original description of the species.

O. MARITIMUS Sacc. Syll. Fung. **2**: 350. 1883.

Halophiobolus maritimus (Sacc.) Linder, Farlowia **1**: 419. 1944.

Linocarpon maritimus (Sacc.) Petr. Sydowia **6**: 388. 1952.

Hab. *Zostera marina,* North Sea.

The spore characteristics of *O. maritimus* given by Saccardo clearly separate this fungus from species of *Lulworthia.* In *O. maritimus,* the ascospore are 140–180 × 10–12 μ, 15–18-septate, breaking into uniseptate segments at maturity. On the basis of this characteristic, as well as in the absence of spore appendages, typical of species of *Lulworthia, O. martimus* is kept in its original genus *Ophiobolus.* However, in the description of *O. maritimus* paraphyses are not reported, while in the genus *Ophiobolus* Reiss, paraphyses are present. It is possible that the paraphyses may deliquesce in *O. maritimus,* since in many of the marine pyrenomycetous species, a deliquescence of the asci has been reported. Type material of this species is not available for examination.

O. salina nom. nov.

O. medusa v. *minor* Ell. & Ev. Proc. Phila. Acad. Nat. Sci. **42**: 239. 1891.

Hab. *Andropogon muricatus,* St. Martinville, Louisiana.

Ellis and Everhart separated *O. medusa* v. *minor* from *O. medusa* on the basis of the larger erumpent ostiole and the smaller asci and ascospores in the former fungus. An examination of the type material of *O. medusa* v. *minor* shows paraphyses present and filamentous ascospores, 90–110 × 2.5 μ, with no appendages. As noted previously, the

species *Lulworthia medusa* (syn. *O. medusa* Ell. & Ev.) is paraphysate and has appendages at the tips of the filamentous spore. From these different descriptions, it is apparent that *O. medusa* v. *minor* is not related to *L. medusa* (syn. *O. medusa* Ell. & Ev.) and warrants recognition as a distinct species.

ZIGNOELLA Sacc.

1. Ascospores less than 150 μ in length.....*Z. calospora*
1. Ascospores more than 150 μ in length.....2
   2. Ascospores 240–250 μ in length.....*Z. cubensis*
   2. Ascospores 280–350 μ in length.....*Z. enormis*

Z. calospora Pat. Jour. de Bot. **11**: 242. 1897.

Hab. *Castagnea chordariaeformis,* Gigon, Spain.

Z. enormis Pat. Jour. de Bot. **17**: 228. 1903.

Hab. *Stypocaulon scoparum,* Spain.

Z. cubensis Hariot & Pat. Bull. Soc. Bot. France **20**: 65. 1904.

Hab. *Stypocaulon scoparum,* Havana, Cuba.

In both *Z. calospora* and *Z. enormis* the perithecia are described as being non-carbonaceous, while in the genus *Zignoella* Sacc., the perithecia are carbonaceous. However, in view of the occurrence in these two species of the characteristic spore and perithecial shape typical of the genus, it does not seem advisable to transfer these species to another genus merely because of differences in the texture of the perithecial wall.

The ascospores of the marine species of *Zignoella* differ from those of *Lulworthia* in being non-appendaged, septate, and with a greater relative width. The width of the spores in the various species of *Zignoella* is from 10–16 μ but never more than 6 μ in *Lulworthia.* The ascospores of *Zignoella* do not fragment at maturity as do those of *Ophiobolus.*

CERATOSPHAERIA Niessl

Ceratosphaeria sp. Johnson, Mycologia **48**: 849. [1956] 1957.

Hab. Yellow poplar panel, Beaufort, North Carolina, September, 1955.

Johnson tentatively places this fungus in the genus *Ceratosphaeria.* The perithecia are black, with a globose venter and a long, black, straight or curved neck. Paraphyses are sparse or lacking, and when present, are short and simple. In the genus *Ceratosphaeria* Niessl, the known

species are paraphysate. The ascospores of this isolate are clavate or cylindrical, hyaline, 2–3-septate, each of the terminal cells with a small hyaline cap. Johnson notes that no species of *Ceratosphaeria* are described as having hyaline "caps" on the end cells of the ascospores.

PHYSALOSPORA Niessl

Physalospora sp. Johnson, Mycologia **48**: 847. [1956] 1957.

Hab. Birch veneer, Beaufort, North Carolina, August, 1955.

Johnson considers the generic position of this isolate of *Physalospora* also tentative, since this fungus has features of both *Sordaria* and *Physalospora*. The ascospores are ovoid, hyaline, non-septate, nonguttulate, surrounded by a hyaline, gelatinous sheath. The latter characteristic is typical of the spores of the genus *Sordaria*, while the color of the ascospores is that of *Physalospora*. The perithecia are globose or subglobose with short, papilliform necks, typical of *Physalospora*. Paraphyses are present.

DOUBTFUL GENERA AND SPECIES

Ceratostomella subsalsa (Crouan) Sacc. Syll. Fung. **1**: 412. 1882.
Sphaeria subsalsa Crouan, Flor. Finistère p. 25. 1867.

Hab. *Atriplex* (*Obione*) sp., Western France.

The perithecia of this species have a long curved neck. The asci are claviform and the ascospores are ovoid, hyaline, with two large granular vacuoles. This incomplete description of the fungus raises doubt as to its correct generic position.

Didymella conchae Bonar, Univ. Calif. Pub. Bot. **19**(5): 188, f. 1, pl. 22. 1936.

Hab. Outer surface of shells of marine animals, *Acmaea, Balanus, Littorina, Mitella, Tegula,* California seashore; shells of *Patella granularia,* Capetown, South Africa.

Santesson (Arkiv Botanik **29A**(10): 47. 1939) notes that this fungus, as well as *Pharcidia marina,* is a synonym of the marine lichen *Arthopyrenia sublitoralis.*

Guignardia gloeopeltidis Miyabe & Tokida, Bot. Mag. Tokyo **61**: 118, f. 1, 1–5. 1948.

Hab. *Gloeopeltis furcata,* Japan.

The ascospores of *G. gloeopeltidis* are described as being definitely

2-celled, with a single transverse septum dividing the spore into two unequal cells at about $\frac{1}{3}$ of the whole length near the attenuated lower end. However, in the genus *Guignardia* Viala & Rav., the spores are 1-celled with a granular band across the center when mature. Thus, this species apparently does not belong in *Guignardia*.

Maireomyces peyssoneliae Feldmann, Bull. Soc. Hist. nat. Afrique du Nord **31**(9) : 166, f. 1. 1940.

Hab. *Peyssonelia squamaria,* Eastern Mediterranean.

The genus *Maireomyces* has several features in common with *Ophiobolus* and *Lulworthia*. In all three genera, paraphyses are lacking and the ascospore is filamentous. The spores of *M. peyssoneliae* are nonseptate, straight or curved, 150–220 × 6–8 μ in size. However, in *Lulworthia* the ascospore has a hyaline appendage at each end, while in *Ophiobolus* the ascospores break into unicellular segments at maturity. Neither of these features is mentioned in the description of the ascospores of *Maireomyces*. Further similarity between the latter genus and *Lulworthia* is in the occurrence of a globose black long-necked perithecia in both genera.

The ascospores of *Maireomyces* are similar to those of *Lulworthia* in their multi-guttulate condition and in the lack of septa. The filamentous spores of *M. peyssoneliae* are broader than are those described for the different species of *Lulworthia*. Feldmann does not mention the presence of appendages in his description of the ascospores of *Maireomyces,* and possibly these were overlooked in his examination of the type material. As previously noted, Ellis and Everhart failed to observe the spore appendages in their description of *Lulworthia* (*Ophiobolus*) *medusa*. Type material of *M. peyssoneliae* has not been examined, and thus I have not been able to ascertain definitely the presence or absence of these appendages. However, the noted similarity between *Lulworthia* and *Maireomyces* warrants placing the latter genus in the list of doubtful taxa until a more complete study of the genus is possible.

Ophiobolus kniepii Bauch, Pub. Sta. Zool. Napoli **15** : 389, f. 1–8. 1936.

Hab. *Lithophyllum expansum, Lithophyllum incrustans* and *Lithophyllum racemus,* Gulf of Naples, Italy.

The ascospores of this species are filamentous, hyaline, 200–260 × 1–2 μ in size. However, the lack of fragmentation of the spore at maturity raises doubt as to the affinity of the species with the genus *Ophiobolus*. The original classification of this species by Bauch was

a provisional one, since, in addition to this discrepancy in ascospore character, the perithecia of *O. kniepii* are definitely long-necked. These necks are from 140–300 μ in length. Except for the lack of appendages in the description of the spores of *O. kniepii*, the filamentous non-fragmenting ascospores and the long-necked perithecia appear to be more characteristic of the genus *Lulworthia*. It is possible that the appendages were overlooked.

Ophiobolus littoralis (Crouan) Sacc. Syll. Fung. **2**: 349.
Sphaeria littoralis Crouan, Flor. Finistère 29. 1867.

Hab. *Agrostis maritima*, Western France.

The perithecia of *O. littoralis* are spherical, black, with a papillate neck. Paraphyses are present. Ascospores are yellow, filiform, 10–12-septate. Spore fragmentation is not mentioned in the original description of the fungus. Since fragmentation of the ascospore at maturity is characteristic of *Ophiobolus*, the inclusion of *O. littoralis* in this genus is open to question.

Pharcidia marina Bommer, Bull. Soc. Belg. Micros. **17**: 151. 1891.

Hab. Barnacle, *Balanus balanoides*.

As mentioned previously, *P. marina* is a synonym of the marine lichen *Arthopyrenia sublitoralis*. Santesson states that this lichen was described in 1889 as the fungus *Ostracoblabe implexa* by Bornet and Flahult (Bull. Soc. Bot. France **36**: 171, pl. 12, f. 1–4. 1889).

Pharcidia pelvetiae Suth. New Phytol. **14**: 39, f. 3, 1–3. 1915.

Hab. *Pelvetia canaliculata*, British Isles.

Sutherland describes *P. pelvetiae* as lacking paraphyses and having 2-celled ascospores that become 4-celled at maturity. Each spore is surrounded by a thin mucilaginous sheath. The rupture of this sheath breaks the spore into two portions. In the genus *Pharcidia* Körb., paraphyses are present and the spores are regularly 2-celled. The dissimilarity between these features of *P. pelvetia* Suth. and those attributed to the genus itself makes the inclusion of this species in *Pharcidia* Körb. questionable.

Physalospora corallinarum (Crouan) Sacc. Syll. Fung. **1**: 448. 1882.
Sphaeria corallinarum Crouan, Flor. Finistère 24. 1867.

Hab. *Corallina officinalis* and *Jania corniculata*, Western France.

Physalospora obionis (Crouan) Sacc. Syll. Fung. **1**: 448. 1882.
Sphaeria obionis Crouan, Flor. Finistère 25. 1867.

Hab. *Atriplex* (*Obione*) sp., Western France.

The perithecia of *P. corallinarum* are black, hemispherical or subconical, lacking a neck. The ascospores are oblong, hyaline and granular. In *P. obionis* the perithecia are black, spherical with a short neck. The asci are long-cylindrical and the ascospores are oblong with large vacuoles. The original description of both of these species is incomplete and no sizes of the asci or ascospores are given. Because of this, the generic position of these two species may be doubtful, and it is difficult even to establish definite species differences for the two fungi.

Stigmea pelvetiae Suth. New Phytol. **14**: 37, f. 2, 1–6. 1915 (as *Stigmatea*).

Hab. *Pelvetia canaliculata*, Scotland.

This species is not referable to *Stigmea* (= *Stigmatea*) Fr., since the spores of *S. pelvetiae* are described as hyaline, whereas the spores of the genus *Stigmea* are brown. The description of *S. pelvetiae* by Sutherland shows closer affinity to the genus *Diplocarpon*.

HOST INDEX [3]

AGLAOZONIA

A. chilosa (= *Cutleria monoica* sec Fritsch)
Melanopsamma Tregoubovii (Oll.) Maire & Oll.
A. parvula (= *Cutleria multifida* sec Fritsch)
Melanopsamma Tregoubovii (Oll.) Maire & Oll.

AGROSTIS

A. maritima
**Ophiobolus littoralis* (Crn.) Sacc.

ANDROPOGON

A. muricatus
Ophiobolus salina Meyers

ASCOPHYLLUM

A. nodosum
Mycosphaerella ascophylli Cotton
Orcadia ascophylli Suth.
Trailia ascophylli Suth.

[3] Asterisk indicates species of fungi from list of doubtful genera and species.

ATRIPLEX (OBIONE)

A. sp.
**Ceratostomella subsalsa* (Crn.) Sacc.
**Didymosphaeria maritima* (Crn.) Sacc.
**Physalospora obione* (Crn.) Sacc.

BRACHYTRICHIA

B. balani
Melanopsamma balani (Winter) Meyers

CASTAGNEA

C. chordariaeformis
Zignoella calospora Pat.

CHONDRUS

C. crispus
Leptosphaeria chondri (Rostr.) Rosen.

CORALLINA

C. officinalis
**Physalospora corallinarum* (Crn.) Sacc.

CYSTOPHORA

C. retroflexa
Melanopsamma cystophorae (Cribb & Herbert) Meyer

CYSTOSEIRA

C. abrotanifolia
Melanopsamma Tregoubovii (Oll.) Maire & Oll.
C. osmundacea
Guignardia irritans Setch. & Estee

DILOPHUS

D. fasciola
Melanopsamma Tregoubovii (Oll.) Maire & Oll.

DILSEA

D. edulis
Mycaureola dilseae Maire & Chemin

FUCUS

F. vesiculosus
Didymosphaeria fucicola Suth.
Lulworthia fucicola Suth.
Orcadia sp.

GLOEOPELTIS

G. furcata
**Guignardia gloeopeltidis* Miyabe & Tokida

HALIDRYS

H. dioica
Guignardia irritans Setch. & Estee

JANIA

J. corniculata
**Physalospora corallinarum* (Crn.) Sacc.

JUNCUS

J. maritimus
Leptosphaeria maritima (Cke. & Plowr.) Sacc.
Leptosphaeria albopunctata (West.) Sacc.
Leptosphaeria marina Ell. & Ev.

LAMINARIA

L. agardhii
Plactostroma laminariae (Rostr.) Meyer
L. digitata
Ophiobolus laminariae Suth.
L. longicruris
Plactostroma laminariae (Rostr.) Meyer
L. saccharina
Hypoderma laminariae Suth.
L. sp.
Pleospora laminariana Suth.
Rosellinia laminariana Suth.

LITHOPHYLLUM

L. expansum
**Ophiobolus kniepii* Bauch
L. incrustans
**Ophiobolus kniepii* Bauch
L. racemus
**Ophiobolus kniepii* Bauch

PELVETIA

P. canaliculata
Didymosphaeria pelvetiana Suth.
Mycosphaerella pelvetiae Suth.
Orcadia pelvetiana Suth.

Plactostroma pelvetiae (Suth.) Meyer
**Pharcidia pelvetiae* Suth.
Pleospora pelvetiae Suth.
**Stigmea* (*Stigmatea*) *pelvetiae* Suth.

PEYSSONELIA

P. squamaria
**Maireomyces peyssoneliae* Feldmann

POSIDONIA

P. oceania
Amphisphaeria posidoniae (Dur. & Mont.) Ces. & DeNot.
Amphisphaeria biturbinata (Dur. & Mont.) Sacc.

PRASIOLA

P. borealis
Guignardia alaskana Reed
P. tessellata
Guignardia prasiolae (Wint.) Reed

SARGASSUM

S. sp.
Phyllachorella oceanica Ferd. & Winge
Guignardia tumefaciens Cribb & Herbert

SPARTINA

S. alterniflora
Leptosphaeria albopunctata (West.) Sacc.
Leptosphaeria discors (Sacc. & Ell.) Sacc & Ell.
Leptosphaeria halima Johnson
Leptosphaeria orae-maris Linder
Leptosphaeria marina Ell. & Ev.
Leptosphaeria maritima (Cke. & Plowr.) Sacc.
Lulworthia medusa (Ell. & Ev.) Cribb & Cribb
Pleospora pelagica Johnson
S. sp.
Leptosphaeria albopunctata (West.) Sacc.
Leptosphaeria discors (Sacc. & Ell.) Sacc. & Ell.
Lulworthia medusa (Ell. & Ev.) Cribb & Cribb

STYPOCAULON

S. scoparum
Zignoella cubensis Hariot & Pat.
Zignoella enormis Pat.

TRIGLOCHIN

T. maritimum
Pleospora maritima Rehm.

ULVA

U. californica
Guignardia ulvae Reed

ZANARDINIA

Z. collaris
Melanopsamma Tregoubovii (Oll.) Maire & Oll.

ZOSTERA

Z. marina
Amphisphaeria posidoniae (Dur. & Mont.) Ces. & DeNot.
Lulworthia halimus (Diehl & Mounce) Cribb & Cribb
Ophiobolus maritimus Sacc.

WOOD AND CORDAGE

Amphisphaeria maritima Linder
Antennospora caribbea Meyers
Arenariomyces quadri-remis Höhnk
Arenariomyces salina Meyers
Arenariomyces trifurcatus Höhnk
Ceratosphaeria sp.
Ceriosporopsis cambrensis Wilson
Ceriosporopsis halima Linder
Ceriosporopsis hamata Höhnk
Halosphaeria appendiculata Linder
Lentescospora submarina Linder
Leptosphaeria albopunctata (West.) Sacc.
Leptosphaeria discors (Sacc. & Ell.) Sacc. & Ell.
Leptosphaeria halima Johnson
Leptosphaeria orae-maris Linder
Lignincola laevis Höhnk
Lulworthia floridana Meyers
Lulworthia fucicola Suth.
Lulworthia grandispora Meyers
Lulworthia medusa (Ell. & Ev.) Cribb & Cribb
Lulworthia medusa var. *biscaynia* Meyers
Lulworthia salina (Linder) Cribb & Cribb
Massariella maritima Johnson

Peritrichospora integra Linder
Peritrichospora lacera Linder
Physalospora sp.
Remispora maritima Linder
Samarosporella pelagica Linder
Sphaerulina orae-maris Linder
Sphaerulina pedicellata Johnson
Sphaerulina sp.
Torpedospora radiata Meyers

LITERATURE CITED

Barghoorn, E. S. and D. H. Linder. 1944. Marine fungi: their taxonomy and biology. Farlowia **1**: 395–467.

Bauch, R. 1936. *Ophiobolus kneipii;* ein neuer parasitischer Pyrenomycet auf Kalkalgen. Pub. Sta. Zool. Napoli **15**: 377–391.

Cotton, A. D. 1907. Notes on marine Pyrenomycetes. Brit. Myc. Soc. Trans. **3**: 92–99.

Cribb, A. B. and J. W. Herbert. 1954. Three species of fungi parasitic on marine algae in Tasmania. Papers, Univ. Queensland Dept. Bot. **3**(2) : 9–13.

—— **and J. W. Cribb.** 1955. Marine fungi from Queensland—I. Papers, Univ. Queensland Dept. Bot. **3**(10) : 77–81.

Höhnk, W. 1954. Studien zur Brack- und Seewassermykologie. IV. Veröff. Inst. Meeresforsch. Bremerhaven **3**: 27–33.

——. 1955. Studien zur Brack- und Seewassermykologie. V. Veröff. Inst. Meeresforsch. Bremerhaven **3**: 199–227.

Johnson, T. W., Jr. 1956. Marine fungi I. *Leptosphaeria* and *Pleospora*. Mycologia **48**: 495–505.

——. [1956] 1957. Marine fungi II. Ascomycetes and Deuteromycetes from submerged wood. Mycologia **48**: 841–851.

Meyers, S. P. 1953. Marine fungi in Biscayne Bay, Florida. Bull. Marine Sci. Gulf and Caribbean **2**: 590–601.

——. 1954. Marine fungi in Biscayne Bay, Florida. II. Further studies of occurrence and distribution. Bull. Marine Sci. Gulf and Caribbean **3**: 307–327.

Ritchie, D. D. 1954. A fungus flora of the sea. Science **120**: 578–579.

Sutherland, G. K. 1915a. New marine fungi on *Pelvetia*. New Phytol. **14**: 33–43.

——. 1915b. Additional notes on marine Pyrenomycetes. New Phytol. **14**: 183–193.

——. 1915c. New marine Pyrenomycetes. Brit. Myc. Soc. Trans. **5**: 147–154.

——. 1916. Additional notes on marine Pyrenomycetes. Brit. Myc. Soc. Trans. **5**: 257–263.

Wilson, I. M. 1951. Notes on some marine fungi. Brit. Myc. Soc. Trans. **34**: 540–543.

——. 1954. *Ceriosporopsis halima* Linder and *Ceriosporopsis cambrensis* sp. nov.: Two marine Pyrenomycetes on wood. Brit. Myc. Soc. Trans. **37**: 272–285.

Zobell, C. E. 1946. Marine Microbiology. Chronica Botanica Co.

THE PERFECT STAGE OF GLIOCLADIUM ROSEUM

EUGENE B. SMALLEY AND H. N. HANSEN

(WITH 12 FIGURES)

Although similarities in asexual characters frequently give clues to relationships, the elucidation of natural relationships among the imperfect fungi always awaits the discovery and study of sexual stages. Thus *Gliocladium* is usually placed near the genus *Penicillium* and some authors (**4**) believe it to be the most closely related of the several similar genera (*Gliocladium, Paecilomyces, Scopulariopsis,* etc.).

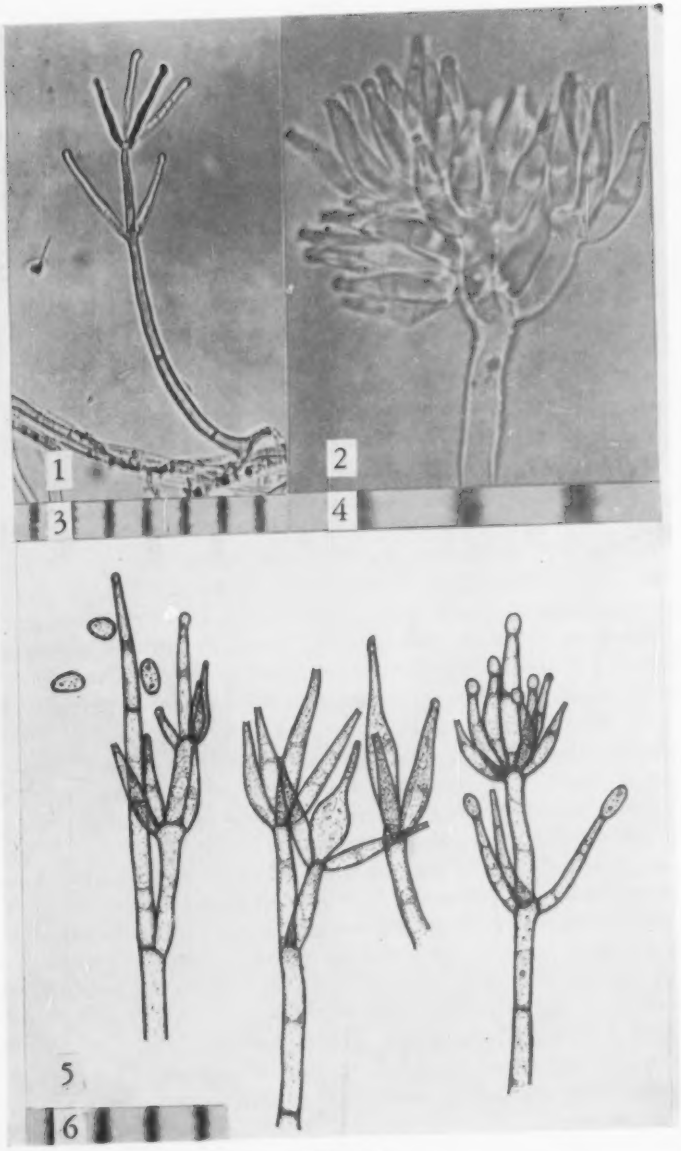
The description of a perfect stage belonging to the order *Eurotiales* of the *Ascomycetes* was published for *Gliocladium* by L. Matruchot (**2**) in 1895. This discovery apparently was never confirmed.

Tulasne (**6**) in his description of *Hypomyces aureo-nitens* Tul. reported that a *Penicillium socium* Tul. was closely associated with this perfect stage. Plowright (**3**) redescribed *H. aureo-nitens,* figured and gave measurements of the same conidial form, but without suggesting the allocation of this to *Penicillium.* Grove (**1**) listed *P. socium* as a synonym for *Gliocladium penicilloides* Corda. The material of both Plowright and Tulasne came from rotting sporophores of fleshy basidiomycetes and involved no pure-culture techniques with which to prove a relationship between this *Hypomyces* and the *Penicillium*-like conidial masses. Thom (**5**) suggested that without further data the name *P. socium* should be dropped, whether or not the organism belonged with *Gliocladium,* as suggested by Grove (**1**).

Raper and Thom (**4**) suggest that *G. penicilloides* is assignable near *G. roseum* (Link) Bainier and is probably synonymous with it.

This paper reports the isolation of a fungus with imperfect characters identical to *G. roseum* and producing a perfect stage assignable to the order *Hypocreales* of the class *Ascomycetes* and to the genus *Nectria.* This fungus was originally isolated in January, 1955, from a decaying bulb of *Lilium auratum* Lindl. When material from this bulb was placed in a moist chamber, perithecia of a *Nectria* sp. developed along with conidial masses of a *Gliocladium* sp.

Water mounts of living material were used in the study of the conidiophores, conidia, asci, and ascospores. Single ascospore cultures



FIGS. 1–6.

obtained from the original perithecia were grown at room temperature (25° C) on 2% potato dextrose agar slants.

Single-conidial cultures from the parent isolates were identified as *Gliocladium roseum*. Their morphological and cultural characters fitted well within the range as given by Raper and Thom (4). Single-ascospore isolates from the parent cultures were compared with a number of isolates of *G. roseum*, including ones from strawberry roots, several from miscellaneous decaying plant material, and the American Type Culture isolate No. *10521*. Culturally there was a certain amount of variation between isolates as to colony appearance and color, but morphologically their conidial characters were identical.

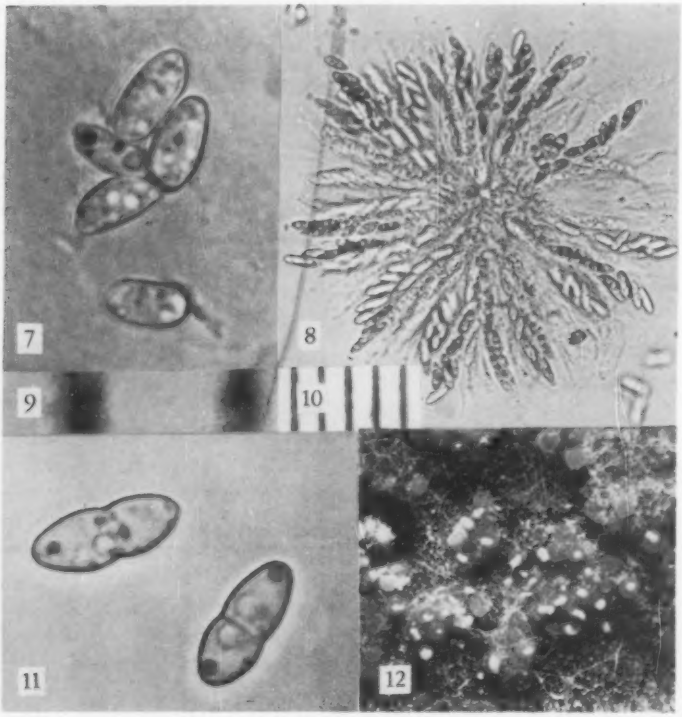
A series of single-ascospore cultures was compared with a similar series of single conidial cultures. The resulting colonies in each case were identical morphologically and culturally, and the fungus homothallic, since both series in time produced perithecia.

The conidial stage of this fungus occurs in two distinct phases. One consists of verticillate conidiophores bearing whorls of elongated sterigmata (FIG. 1). For reference this will be called the *Verticillium* phase. The other consists of conidiophores bearing the "penicillus" or brush-shaped conidium-bearing apparatus typical of the genus *Gliocladium* (FIG. 2). For reference this will be called the *Gliocladium* phase. On 2% water-agar plates the *Verticillium* phase develops shortly before the *Gliocladium* phase, and both produce conidia in mucilaginous drops. When grown on a richer medium (PDA) both phases appear at nearly the same time. On the richer medium the conidia adhere to form chains, but these often break up into mucilaginous masses as the cultures age. In these older cultures additional intermediate conidial phases can be found which form a transition between the *Verticillium* and *Gliocladium* phases (FIG. 5). This appears to be the result of a shortening of both sterigmata and the "internodal" cells between the sterigmatal whorls of the *Verticillium* phase. Thus the *Verticillium* phase may alter gradually to become the *Gliocladium* phase (FIG. 5).

On PDA slants at room temperature the perithecia are usually formed after two months although this varied somewhat with the isolate. The

FIGS. 1–6. *Nectria gliocladioides*. 1. Conidiophore of the imperfect stage, *Gliocladium roseum*, showing the *Verticillium* phase. 2. Upper portion of the conidiophore of *G. roseum* showing the *Gliocladium* phase. 3. 0.01 mm divisions of stage micrometer to indicate the scale of Fig. 1. 4. 0.01 mm divisions of the stage micrometer to indicate the scale of Fig. 2. 5. Drawing made from photographs of conidiophores of *G. roseum* showing intermediates between the *Verticillium* and *Gliocladium* phases. 6. 0.01 mm divisions of stage micrometer to indicate scale of Fig. 5.

perithecia are spherical to obovate and are borne densely clustered on fleshy yellow stromata or scattered without stromata (FIG. 12). They are light yellow-orange in color, the walls somewhat warty in appearance and free of hairs or bristles. The ostioles are inconspicuous, nonpapilliform and with numerous fine periphyses on the inner surfaces.



FIGS. 7–12. *Nectria gliocladioides*. 7. Conidia of the imperfect stage. 8. Showing general shape, ascospores and ascospore arrangement. 9. 0.01 mm divisions of stage micrometer to indicate scale of Figs. 7, 11. 10. 0.01 mm divisions of stage micrometer to indicate scale of Fig. 8. 11. Ascospores, showing median septations and constrictions at the septa. 12. Perithecia, × 10.

The asci are clavate-cylindrical and contain 8 obliquely uniseriate ascospores (FIG. 8). The ascospores are elliptical, hyaline, uniseptate, and constricted at the septum (FIG. 11). No paraphyses are present.

Although several species of *Nectria* and *Hypomyces* have been described with *Verticillium*-like imperfect stages (*Nectria vulgaris* Speg.,

Hypomyces aureo-nitens Tul., *H. broomeanus* Tul., *H. ochraceus* Tul., *H. terrestris* Plowr. & Boud., etc.) none, with the possible exception of *H. aureo-nitens,* have been reported with a *Gliocladium* imperfect stage. On this basis a new species of *Nectria* is established.

Nectria gliocladioides sp. nov.

Peritheciis liberis, caespitosis vel discretis, stromate pulviniformi, sphaericis vel obovatis, aureo-flavis, 180–260 × 200–280 μ; ostiolo inconspicuo; ascis cylindraceo-clavatis, octosporis, 61–69 × 4–8 μ; aparaphysatis; ascosporidiis uniseriatis, hyalinis, uniseptatis, constrictis, plerumque 10–12 × 3–4 μ. Status conidicus: *Gliocladium roseum* (Link) Bainier.

The type is represented by a single-spore culture originally isolated from a decaying bulb of *Lilium auratum* in January, 1955. Material from this type has been placed in the American Type Culture Collection, Washington, D. C., and the Centraalbureau voor Schimmelcultures, Baarn, Holland.

This fungus has its closest natural relationships with other *Nectria* and *Hypomyces* species having verticillate or subverticillate imperfect stages and is unrelated to *Penicillium,* although having similar imperfect morphological characters.

SUMMARY

This paper describes the isolation and identification of the perfect stage of *Gliocladium roseum* (Link) Bainier. The perfect stage is characterized as a homothallic fungus assignable to the order *Hypocreales* of the *Ascomycetes* and to the genus *Nectria.* The species has been designated as *Nectria gliocladioides.*

DEPARTMENT OF PLANT PATHOLOGY
UNIVERSITY OF CALIFORNIA
BERKELEY 4, CALIFORNIA

LITERATURE CITED

1. **Grove, W. B.** 1885. New or noteworthy fungi. Jour. Bot. (London) **23**: 129–134; 161–169.
2. **Matruchot, L.** 1895. Structure, developpement et forme parfaite des *Gliocladium.* Rev. Gen. de Bot. **7**: 321–331.
3. **Plowright, C. B.** 1882. A monograph of the British Hypomyces. Grevillea **11**: 4–8; 41–51.
4. **Raper, K. B. and Charles Thom.** 1949. A Manual of the Penicillia. Pp. 1–874. The Williams and Wilkins Co., Baltimore, Md.
5. **Thom, C.** 1930. The Penicillia. Pp. 1–643. The Williams and Wilkins Co., Baltimore, Md.
6. **Tulasne, L. R. and C. Tulasne.** 1861–1865. Selecta fungorum carpologia. V. 3, p. 64. Typographie Imperiale, Paris.

STUDIES IN CONIOPHORA. I. THE BASIDIUM

PAUL L. LENTZ

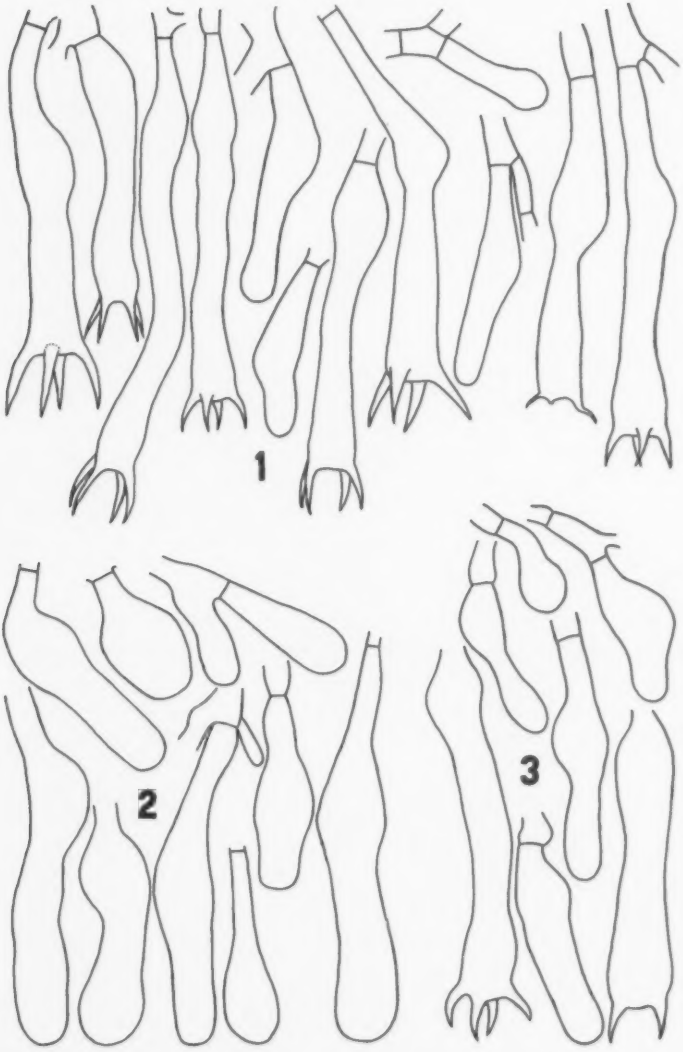
(WITH 21 FIGURES)

Coniophora DC. ex Mérat is a genus of the Thelephoraceae characterized by effused habit, essentially smooth hymenial surface, and smooth, colored spores that have peg-like apiculi. The genus has economic importance because of the wood-destroying propensities of some species, notably *C. puteana* (Schum. ex Fr.) Karst. There are probably not more than 20 or 30 species, but the taxonomy of the genus is in such state as to make an accurate estimate difficult. Burt (**1917, 1926**) included in *Coniophora* several species that have since (Rogers and Jackson, **1943**) been excluded even though they have colored spores. The nature of the hyphae and the presence or absence of cystidia have been used along with spore-character as a basis for generic delineation. The basidium of *Coniophora* has essentially been ignored.

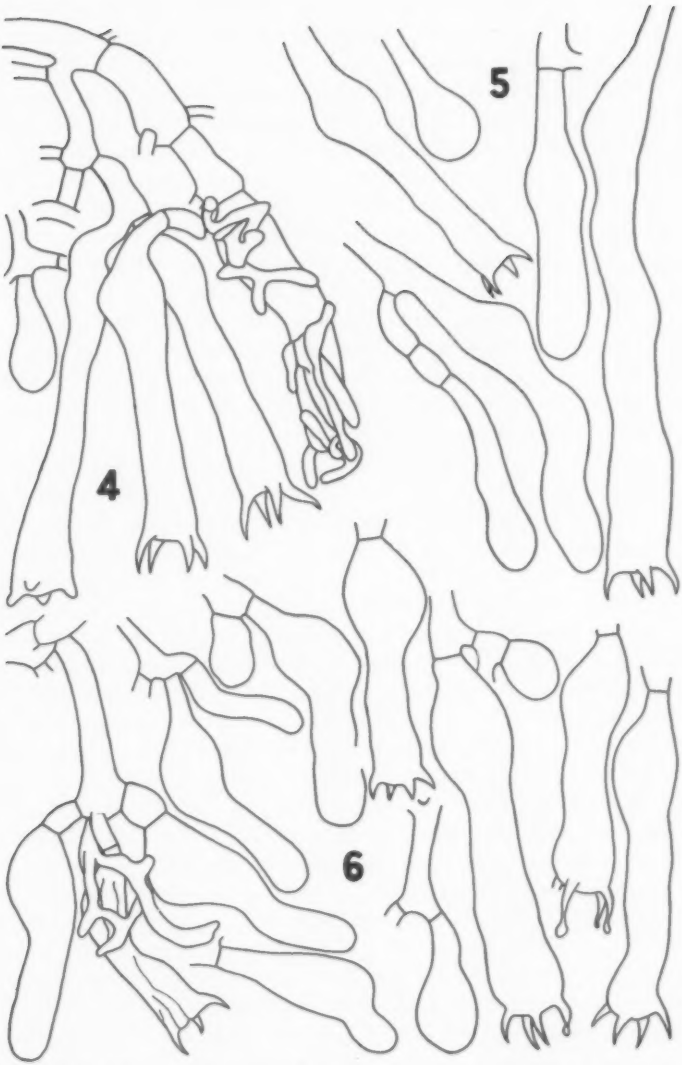
Among many basidiomycetes, the basidium has been shown to have anatomical modifications that may provide clues to taxonomic relationships. Examples are the basidia of *Trechispora* and *Galzinia* (Rogers, **1944**; Olive, **1954**). Although somewhat less attention has been directed toward the basidia of many other genera among the more primitive homobasidiomycetes, some in addition to *Trechispora* and *Galzinia* may have basidia that depart considerably from the typical clavate development. Among these are *Vararia, Aleurodiscus, Asterostroma, Tomentella,* and some species of *Corticium* and *Peniophora. Coniophora* has a basidiocarp that lacks involved hyphal patterns. In this sense it may be said to resemble most of these other genera in having a corticioid type of fructification. In fungi with this simple kind of construction, the basidium is one of relatively few anatomical features that affords a possibility of fruitful study. In order to determine whether the basidia of *Coniophora* are distinctive, more than 100 specimens in the National Fungus Collections have been examined. The present condition of taxonomy in the genus does not permit entirely accurate determination of species in all instances, but that has relatively little importance in this study. Those specimens that may not be determined with absolute certainty are mostly in the complex centered around *C. arida* (Fr.) Karst.

In *Coniophora* the basidium originates as a very small, more or less cylindrical, apical cell in the basidial fascicle. The cell soon enlarges laterally to become subglobose or pyriform. Often the basal part of this cell may remain narrow and form a stalk for the enlarged apical part. The next stage of development is most significant, for it shows the difference between the basidium of *Coniophora* and the more usual kind of homobasidiomycetous basidium. In this stage the original enlargement, or vesicle, does not lose its structural integrity but merely has protruded from its apex a tubular outgrowth of smaller diameter than that of the vesicle. (This development is shown in nearly all of the figures accompanying this discussion, but the stages discussed up to this point are demonstrated especially well by FIG. 2.) The tubular outgrowth continues to elongate. At this stage the basidium may bear some resemblance to the non-septate young basidium of the Auriculariaceae. Eventually the elongated tube is surmounted by four, or more rarely two, apical sterigmata. This ontogeny, the protrusion of a tube from the apex of a basal vesicle, is different from that characteristic of the typical clavate basidium, which usually develops merely by the progressive elongation and slight apical enlargement of an originally cylindrical basidiole. (Variations of clavate basidia are illustrated by FIGS. 16–20.) The development of the *Coniophora* basidium is all the more unusual because the basal enlargement remains evident when the basidium is mature. The enlargement is sometimes difficult to discern, but apparently is never lacking after it is first formed early in the development of the basidium.

Occasionally there may be minor variations of the characteristic form described, but in the typical species these are not of a nature that will alter the basic pattern. The vesicle may be more elongated or narrower than usual, but the development is as previously described. In many specimens of *C. puteana, C. arida,* and related species there is a pronounced tendency for all context and hymenial elements to assume a flat, waxy, characterless appearance when mounted in KOH or Melzer's solution. The basidia of these specimens may swell unduly and are then unsuitable for study. This characteristic seems often to be associated with a waxy appearance of the hymenial surface. The basidia of these specimens must be examined soon after immersion in the mounting fluid in order to minimize distortion. In some specimens the apical tube may be greatly elongated, and there is a tendency for it to break off just above the basal vesicle. Often the basal region of the basidium is concealed by a profuse development of slender, much-branched hyphae (FIGS. 4, 6, 8). All of these factors complicate the



FIGS. 1–3. Basidia of *Coniophora*. 1. *C. olivacea*. 2. *C. cerebella* (*puteana*). 3. *C. flavobrunnea*. All × 1000.

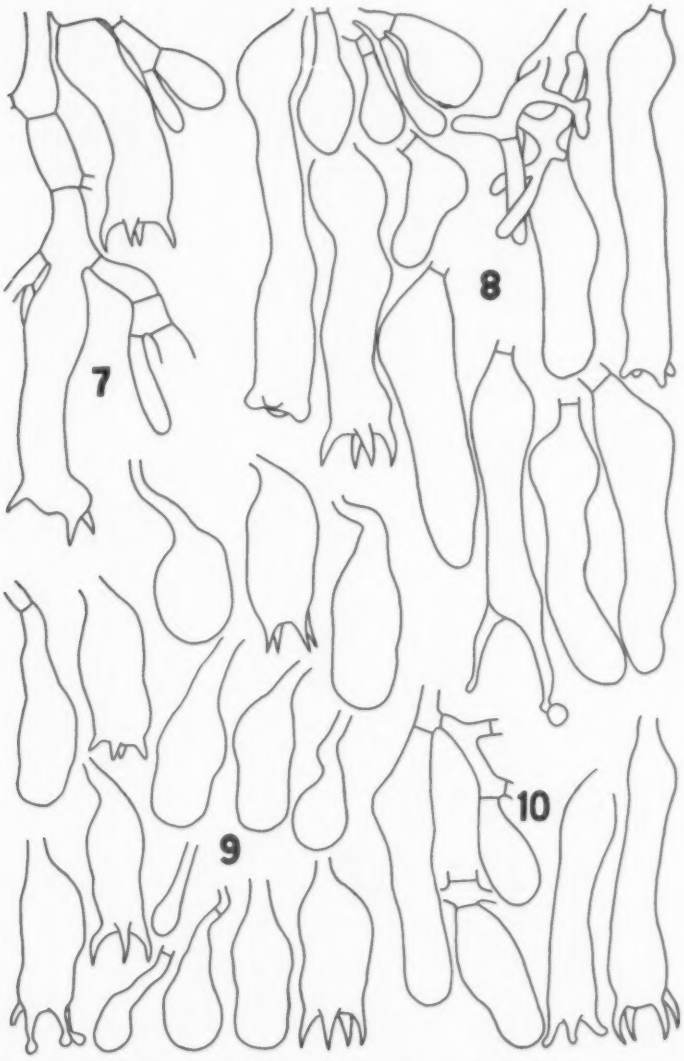


FIGS. 4–6. Basidia of *Coniophora*. 4. *C. flavobrunnea*. 5. *C. laxa*. 6. *C. kalmiae*. All × 1000.

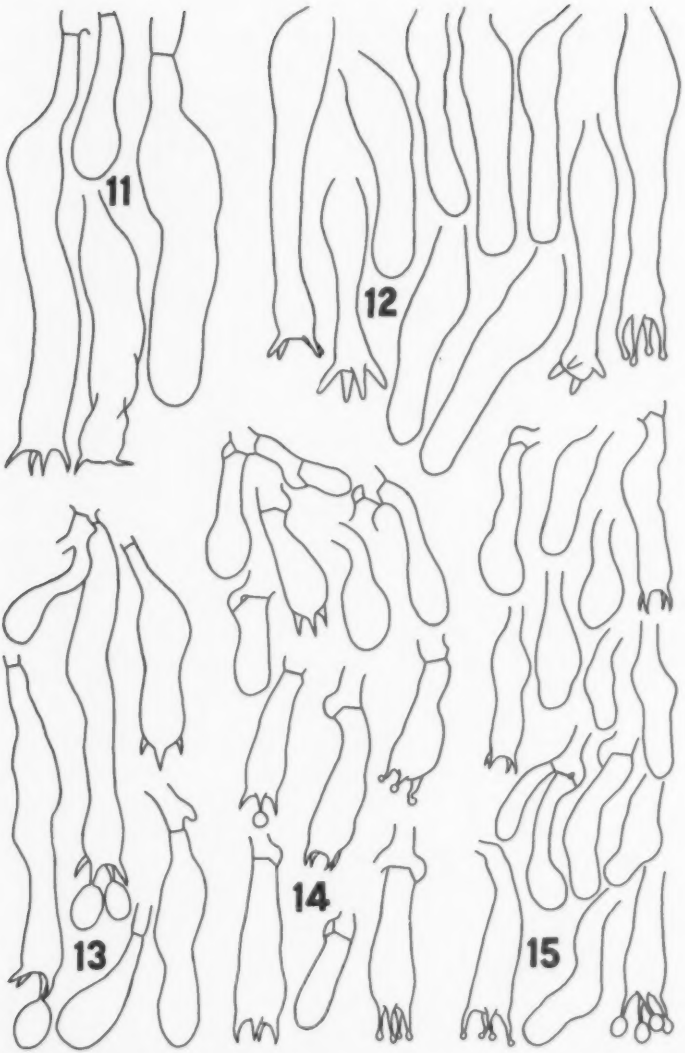
interpretation of basidial form. In most specimens the younger, marginal areas of the hymenium provide basidia that are more satisfactory for study than those in older areas.

The distinctive nature of the *Coniophora* basidium is accentuated by its nuclear activity. In the very young basidiole (FIG. 21, A–D), two nuclei may be seen when stained with ferric aceto-carmine. Shortly after the vesicle starts to form, these nuclei fuse (FIG. 21, E). The fusion nucleus stays in the basal vesicle while the tube begins to develop (FIG. 21, G–I), and it remains in the swollen basal region until the basidium has practically completed its elongation. Then the nucleus moves into the tube (FIG. 21, K–M) and divides when it approaches the apex of the basidium (FIG. 21, N). At this stage the contents of the tube are very obscure, and a second nuclear division has not been observed. The interesting aspect of this nuclear activity is the long delay of the nucleus in entering the apical tube and the postponement of division until it does migrate into this tube.

Certain species present unusual difficulty in the interpretation of the basidium. Among these are *C. alboflavescens* (Ell. & Ev.) Höhn. & Litsch. and *C. olivascens* (Berk. & Curt.) Mass. The basidia of *C. alboflavescens* (FIG. 9) are short and broad, with little lengthening of the apical protrusion. However, the development of these basidia seems homologous with that in other species of *Coniophora*. The spores of this species are globose, but otherwise similar in structure to the spores typical of *Coniophora*. Thus there seems to be no reason to doubt that *C. alboflavescens* is truly a *Coniophora*. The interpretation of *C. olivascens* is more difficult. In this species the basidia and spores are considerably smaller than those in most species of *Coniophora*. The apiculus of the spore is often relatively small but seems to have somewhat the peg-like aspect found in spores of *Coniophora*. Often the spores have a greenish tinge. In most specimens examined, the basidia tend to be of the clavate type, with fewer of the *Coniophora* type (FIG. 14). In two specimens from Idaho the basidia were much more generally of the *Coniophora* type, with fewer apparently of the clavate type (FIG. 15). These two specimens differed from the other specimens of *C. olivascens* also by the fact that their spores assumed a very dark blue color upon contact with KOH solution, whereas the spores of other specimens remained olivaceous. The two Idaho specimens are apparently *C. mustialaensis* (Karst.) Mass. instead of *C. olivascens*. The spores are pale yellowish green when mounted in water and do not assume the dark blue color until they come into contact with KOH solution. If a crushed mount is made in water and KOH solution is

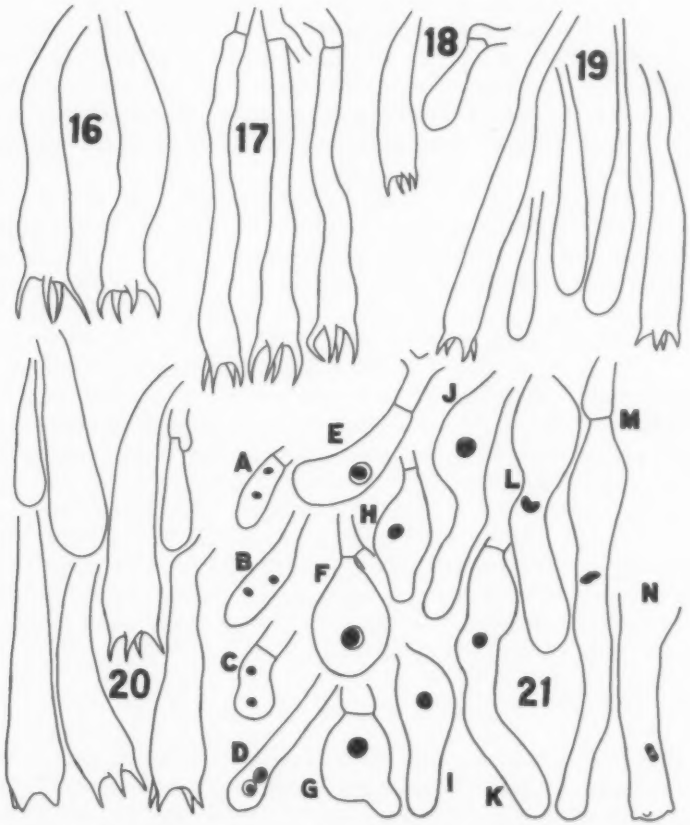


FIGS. 7–10. Basidia of *Coniophora*. 7. *C. kalmiae*. 8. *C. arida*. 9. *C. albo-flavescens*. 10. *C. sistotremoides* (*suffocata*). All × 1000.



FIGS. 11–15. Basidia of *Coniophora*. 11. *C. fusispora*. 12. *C. papillosa*. 13. *C.* sp., from basidiocarp produced in culture. 14. *C. olivascens*. 15. *C. "olivascens"* (*C. mustialaensis*?). All ×1000.

subsequently run in under the cover slip, only those spores in the marginal area of the smear are likely to turn blue. If the cover slip is then removed, all of the spores instantly turn blue. This color reaction of the spores to KOH solution is similar to that described for *Amaurodon viridis* sensu Schroet. (1888, p. 461), which Rogers and Jackson (1943) believe to be synonymous with *C. mustialaensis*. In general aspect, hyphal character, and small size of basidia and spores



FIGS. 16–21. Basidia of *Coniophora, Pellicularia, Peniophora,* and *Serpula.* 16. *C. ochroleuca* (*Pellicularia ochroleuca*). 17. *C. laeticolor* (*Peniophora dryina*). 18. *S. eurocephala.* 19. *S. pinastri.* 20. *S. lacrimans.* 21. *C. kalmiae,* showing stages of nuclear activity. All × 1000.

the other specimens determined as *C. olivascens* are similar to those believed to be *C. mustialaensis*. However, the basidia seem to be more nearly the clavate type than the *Coniophora* type, and the apiculus of the spore is not so peg-like as in the other two specimens. This species, as represented by eleven specimens examined, may be a *Corticium* or *Peniophora* instead of a *Coniophora*.

Some other specimens included as *Coniophora* in the National Fungus Collections do not seem to belong in the genus. In most instances these have neither the basidia nor the spores of a *Coniophora*. Among those examined are specimens of *C. byssoides* (Fr.) Karst., *C. corrugis* Burt, *C. dryina* (Berk. & Curt.) Mass., *C. lacticolor* (Karst.) Karst. (FIG. 17), and *C. ochroleuca* Bres. (FIG. 16). Rogers (**1943**) has placed *C. ochroleuca* in *Pellicularia*. Rogers and Jackson (**1943**) have discussed the other species and have excluded all from *Coniophora*.

The suggestion has been made (cf. the arrangement in Bourdot and Galzin, **1928**) that *Coniophora, Coniophorella,* and the brown-spored species of *Merulius* [= *Serpula*] may be closely related. *Coniophorella* is based on *Coniophora olivacea* (Fr.) Karst. (FIG. 1). Although this species has cystidia, all other morphological aspects are those of *Coniophora* and there does not seem to be sufficient reason for removing it from this genus. *Serpula* has been held to differ from *Coniophora* primarily because it has an alveolate, instead of even, hymenial surface. The basidia from specimens of three *Serpula* species have been examined to see whether their development approximates that in *Coniophora*. These specimens had previously been determined by Dr. W. B. Cooke as *S. eurocephala* (Berk. & Br.) W. B. Cke. (FIG. 18), *S. lacrimans* Pers. ex S. F. Gray (FIG. 20), and *S. pinastri* (Fr.) W. B. Cke. (FIG. 19). In each instance the basidial development has been found to be the cylindrical-clavate type common among the homobasidiomycetes. In this respect, at least, these species of *Serpula* do not seem to exhibit a very close relationship to *Coniophora*.

SPECIES STUDIED AND ILLUSTRATED

The specimens examined were filed in the National Fungus Collections under the following names: *Coniophora alboflavescens* (Ell. & Ev.) Höhn. & Litsch. (FIG. 9), *C. arida* (Fr.) Karst. (FIG. 8), *C. atrocinerea* Karst. [= *C. olivacea*], *C. betulae* (Schum. ex Pers.) Karst., *C. byssoidea* (Fr.) Karst. [= *Peniophora byssoides* (Fr.) Bres.], *C. cerebella* Pers. (FIG. 2) [= *C. puteana*], *C. corrugis* Burt [= *Cor-*

ticium corruge (Burt) Burt ex Rogers & Jacks.], *C. dryina* (Berk. & Curt.) Mass. [= *Peniophora dryina* (Berk. & Curt.) Rogers & Jacks.], *C. ellisii* (Berk. & Cke.) Sacc. [= *C. olivacea*], *C. flavobrunnea* Bres. (FIGS. 3, 4), *C. fuscata* Bres. & Torrend, *C. fusispora* (Cke. & Ell.) Cke. (FIG. 11), *C. kalmiae* (Pk.) Burt (FIGS. 6, 7, 21), *C. laeticolor* (Karst.) Karst. (FIG. 17) [= *Peniophora dryina*], *C. laxa* Fr. (FIG. 5), *C. leucothrix* (Berk. & Curt.) Sacc. [= *C. olivacea*], *C. lurida* (Karst.) Bres., *C. macra* Karst., *C. membranacea* DC. ex Mérat [= *C. puteana*], *C. ochroleuca* Bres. (FIG. 16) [= *Pellicularia ochroleuca* (Bres.) Rogers], *C. olivacea* (Fr.) Karst. (FIG. 1), *C. olivascens* (Berk. & Curt.) Mass. (FIGS. 14, 15), *C. papillosa* Talbot (FIG. 12), *C. prasina* (Berk. & Curt.) Höhn. & Litsch. [= *C. olivascens*], *C. prasinoides* Bourd. & Galz., *C. puteana* (Schum. ex Fr.) Karst., *C. sistotremoides* (Schw. ex Fr.) Mass. sensu Burt, **1917** (FIG. 10) [= *C. suffocata*], *C.* sp. (FIG. 13), *C. subcinnamomea* Karst., *C. suffocata* (Pk.) Mass., *Serpula eurocephala* (Berk. & Br.) W. B. Cke. (FIG. 18), *S. lacrimans* Pers. ex S. F. Gray (FIG. 20), *S. pinastri* (Fr.) W. B. Cke. (FIG. 19).

SUMMARY

1. More than 100 specimens filed as *Coniophora* in the National Fungus Collections have been examined in order to determine the developmental morphology of the basidium. These specimens, according to the identifications accepted as reasonably satisfactory, were distributed among 30 species.

2. In all instances the basidial development in species characteristic of *Coniophora* has been found to involve the production of an enlarged basal vesicle with subsequent protrusion of a more slender tube from the apex of the basal enlargement. Sterigmata are then formed at the apex of the tube.

3. In the young basidiole two nuclei are observed. These fuse as the basidiole enlarges. The fusion nucleus remains in the basal vesicle until the apical tube has elongated nearly to its ultimate length. The nucleus then migrates into the tube and divides as it nears the apex of the basidium.

4. *C. alboflavescens* departs from the typical aspect by the failure of the apical protrusion to elongate much, but in all other respects the development in this species is homologous with that in other species.

5. *C. olivascens,* as represented by specimens in the National Fungus Collections, has smaller spores and basidia than typical species of *Coniophora* and the development of the basidium is variable between

the *Coniophora* type and the clavate type. Two of the specimens examined are probably *C. mustialaensis*. The others appear to represent *C. olivascens*, but this species may be placed more properly in *Corticium* or *Peniophora*.

6. *C. olivacea* differs from other species of *Coniophora* by having cystidia, but in all other respects is a typical *Coniophora* and should not be a basis for maintaining the genus *Coniophorella*.

7. Although they have spores similar to those of *Coniophora*, specimens of three species of *Serpula* have been found to have clavate basidia and thus do not show a very close relationship to *Coniophora* in this respect.

NATIONAL FUNGUS COLLECTIONS
PLANT INDUSTRY STATION
BELTSVILLE, MARYLAND

LITERATURE CITED

Bourdot, H. and A. Galzin. 1928. Hyménomycètes de France. Sceaux.

Burt, E. A. 1917. The Thelephoraceae of North America. VIII. *Coniophora*. Ann. Missouri Bot. Gard. **4**: 237–269.

——. 1926. The Thelephoraceae of North America. XV. (Conclusion with supplement and general index.) *Coniophora*. Ann. Missouri Bot. Gard. **13**: 310–312.

Olive, L. S. 1954. Two species of *Galzinia* from the southern Appalachians. Mycologia **46**: 794–799.

Rogers, D. P. 1943. The genus *Pellicularia* (Thelephoraceae). Farlowia **1**: 95–118.

——. 1944. The genera *Trechispora* and *Galzinia* (Thelephoraceae). Mycologia **36**: 70–103.

—— **and H. S. Jackson.** 1943. Notes on the synonymy of some North American Thelephoraceae and other resupinates. Farlowia **1**: 263–328.

Schroeter, J. 1888. Die Pilze Schlesiens. *In F. Cohn*, Kryptogamen-Flora von Schlesian **3**(1), Fasc. 4.

VOLVARIELLA IN NORTH AMERICA [1]

ROBERT L. SHAFFER

(WITH 15 FIGURES)

Volvariella is a sharply circumscribed group of agaric species characterized macroscopically by possessing pink spores, free lamellae, and a stipe which bears no annulus but is encased at the base by a volva. The genus is widely distributed; species have been reported from tropical, subtropical, and temperate regions of both the eastern and the western hemispheres.

More than one hundred species, subspecies, and varieties, which today would be considered to belong in *Volvariella,* have been described from throughout the world. Eighteen species are here recognized for North America. Lloyd (**1898**, **1899a**, **1899b**, under the name *Volvaria*) and Murrill (**1917**, under the name *Volvariopsis*) were the last to treat the North American species of the genus, but Kauffman (**1918**, under the name *Volvaria*) and Coker (**1947**, under the name *Volvaria*) published keys to and descriptions of the species occurring in Michigan and North Carolina, respectively.

THE GENERIC NAME

The genus treated in this paper has been known by four names since the starting point of nomenclature for the agaricaceous fungi. The Latin word "volva" (a wrapper, covering, or integument) is the root for three of these names: *Volvaria, Volvariopsis,* and *Volvariella;* the fourth is *Pseudofarinaceus.*

The name best known for the genus is *Volvaria.* Fries (**1821**) used this for his "tribe" containing pink-spored, volvate agarics with free

[1] This paper is based upon a Ph.D. thesis submitted to the Graduate School of Cornell University in August, 1955. The author wishes to express his thanks to Dr. Richard P. Korf of the Department of Plant Pathology, Cornell University, who directed the study, and to the United States Public Health Service and the National Science Foundation for granting fellowships which made the author's graduate program possible. Work on *Volvariella* has been continued in the Department of Botany, University of Chicago, Chicago 37, Illinois, the author's present address.

lamellae, and Kummer (**1871**) raised *Volvaria* to generic rank.[2] However, De Candolle (in Lamarck and De Candolle, **1805**) had previously described a lichen genus which he named *Volvaria*. The fact that *Volvaria* DC. 1805 antedates *Volvaria* (Fr.) Kummer 1871 [or (Fr.) Quél. 1872, as the author citation was given before the recent rediscovery of Kummer's work] apparently led Earle (**1909**) to resurrect *Pseudofarinaceus* Batt. 1785 for the agaric genus. This name, however, had been taken up by Kuntze in 1891 for the white-spored genus now known as *Amanitopsis* Roze. Murrill (**1911**) then published the name *Volvariopsis* for the pink-spored genus.

Proposals to conserve *Volvaria* (Fr.) Kummer 1871 [or (Fr.) Quél. 1872] against *Pseudofarinaceus* Batt. ex Earle 1909 (Maire in Briquet, **1935**; Wakefield, **1939**) and against *Volvariopsis* Murr. 1911 (Singer and Smith, **1946**) were unnecessary, but those made for conservation against *Volvaria* DC. 1805 (Wakefield, **1939**; Donk, **1941, 1949**) were appropriate. One point stressed in favor of conservation was that *Volvaria* DC. appeared to be a *nomen ambiguum* and had not been used by lichenologists since 1859. Singer and Smith (**1946**) considered conservation not to be actually necessary since Murrill had made most of the important transfers to *Volvariopsis*. Rogers (**1949**) recommended that any proposal to conserve *Volvaria* (Fr.) Kummer be rejected because conservation would not "avoid disadvantageous changes" in nomenclature (Article 21, International Rules of Botanical Nomenclature, ed. 3).

Donk (**1949**) was apparently the first to suggest that the use of *Volvariopsis* was endangered by *Volvariella* Speg. Spegazzini described *Volvariella* (type: *V. argentina* Speg.) in 1899 and considered it to differ from the agaric genus *Volvaria* in having a cartilaginous stipe and the pileus and stipe continuous. These differences do not warrant the recognition of a separate genus, and the type species is a true *Volvaria* in the sense of modern authors (*e.g.*, Patouillard, **1900**; Singer, **1950**). Because *Volvariella* Speg. 1899 would replace *Volvariopsis* Murr. 1911, Rogers (**1950**) recommended that *Volvaria* (Fr.) Kummer

[2] Singer (**1951b, 1955**) was convinced that Kummer did not propose a new status for Friesian "tribes" but rather new genera coinciding with the "tribe" names of corresponding groups in Fries' works. If this is so, the correct citation here would be *Volvaria* Kummer and not *Volvaria* (Fr.) Kummer. Although nothing of the sort appears in the first edition, in the second edition of *Der Führer in die Pilzkunde* (**1882**) Kummer makes this statement on the second page of the foreword: "In der systematischen Anordnung habe ich mich aus praktischen Gründen im Allgemeinen an Elias Fries gehalten." This statement plus the correspondence of Fries' "tribes" and Kummer's genera seems to be good argument for the "(Fr.) Kummer" manner of citation.

be conserved against *Volvaria* DC. in order to avoid an entirely new set of binomials (*i.e.*, in *Volvariella*). A proposal for this conservation was rejected by the Special Committee for Fungi of the Nomenclature Section of the International Botanical Congress (Rogers, **1953**).

SYSTEMATIC ACCOUNT

The general procedure outlined by A. H. Smith (**1949**) was used in studying dried herbarium material. In a species description, spore size is based upon measurements of 50, 20, or 10 spores selected randomly from each specimen examined. For each species the total range of each dimension is given, and this is followed, in parentheses, by the range of the average dimension, which was calculated for each specimen. Q is the length/width ratio of the spores. The length of the spores includes the apiculus.

Descriptions of size and shape of basidia and cystidia are based upon 20 observations on each specimen in the case of type specimens and 10 observations in the case of all other specimens examined. The length of the sterigmata is included in basidium length.

The symbol (!) after a binomial listed in synonymy indicates that the type specimen of that species has been examined. The recommendations of Stevenson (**1953**) for the abbreviations of the names of authors have been followed. The information concerning specimens examined, when known, is given in the order "Collector and no., Locality, Date (Herbarium where deposited and no.)." Symbols for herbaria are those of the *Index Herbariorum* (Lanjouw and Stafleu, **1956**) except that "RLS" denotes the author's personal herbarium.

VOLVARIELLA Speg.

An. Mus. nac. B. Aires **6**: 118. [1899.]

Agaricus "tribe" *Volvaria* Fr., Syst. Myc. **1**: 277. 1821.

Volvaria (Fr.) Kummer, Führ. Pilzk. 23. 1871. Non *Volvaria* DC. in Lam. & DC., Flore Franç., ed. 3, **2**: 373. 1805.

Pseudofarinaceus Batt. ex Earle, Bull. N. Y. Bot. Gard. **5**: 449. 1909. Non *Pseudofarinaceus* Batt. ex Kuntze, Rev. Gen. Plant., part 2, p. 867. 1891.

Volvariopsis Murr., Mycologia **3**: 280. 1911.

Carpophores fleshy; small, medium, or large in size. Lamellae free. Stipe central, with no annulus but with a well developed volva at the base. Spores smooth, not truncate, with moderately thickened walls, non-amyloid, dull pink to rose or brownish pink in mass. Basidia clavate, usually 4-spored. Cystidia usually present and prominent.

Subhymenium pseudoparenchymatous. Lamella trama inverse. Pileus trama of branched, interwoven hyphae. Pileus cuticle of more or less radially arranged hyphae, gelatinous or not. Clamp connections usually absent.

Habitat on soil, wood, dung, or other agarics.

TYPE: *Volvariella argentina* Speg.

KEY TO SPECIES

In the following key the names of accepted species are in small capitals; those of imperfectly known species, in italics.

A. Spores > 11 μ long, > 7 μ broad (B)
  B. Pileus not viscid, *i.e.*, without a culticle of gelatinized hyphae (C)
    C. Pileus white or pale gray (D)
      D. Stipe furrowed for its entire length; spores 15.7–22.9 × 8.7–11.4 μ; pleurocystidia fusoid ventricose, common.....1. VOLVARIELLA CANALIPES
      D. Stipe not furrowed; spores 11.2–15.4 × 6.9–9.7 μ; pleurocystidia clavate to ovoid, rare.....2. VOLVARIELLA EARLEI
    C. Pileus avellaneous to brown (E)
      E. Pileus 6–7 cm broad, spores 14.7–20.3 × 8.6–10.7 μ.....3. VOLVARIELLA ALABAMENSIS
      E. Pileus 1–5 cm broad, spores 9–12 × 5–7 μ (Kauffman, **1924**).....19. *Volvaria avellanea* and 21. *Volvaria concinna*
  B. Pileus viscid, *i.e.*, with a cuticle of gelatinized hyphae (F)
    F. Pileus 3–5 cm broad; stipe slender, 0.5–0.8 cm thick, glabrous; spores 11.2–15.4 μ long; pleurocystidia ovoid to clavate, rare....2. VOLVARIELLA EARLEI
    F. Pileus 5–15 cm broad; stipe stout, (0.5–)1–2 cm thick, tomentose or villose at least at the base; spores 11.2–20.9 μ long; pleurocystidia of diverse shapes, usually fusoid-ventricose or clavate, common (G)
      G. Pileus white or cream color, occasionally avellaneous to light brownish gray, with the margin not striate or only slightly so.....4a. VOLVARIELLA SPECIOSA var. SPECIOSA
      G. Pileus fulvous to fuliginous or pearl gray, occasionally whitish, with the margin striate.....4b. VOLVARIELLA SPECIOSA var. GLOIOCEPHALA
A. Spores < 11 μ long, < 7 μ broad (H)
  H. Pileus viscid, fulvous-ochraceous; stipe and volva ochraceous.....23. *Volvaria viscosa*
  H. Pileus not viscid or if viscid, then white; carpophore not ochraceous overall (I)
    I. Pileus 5–20 cm broad or, if smaller, then bright yellow; habitat on wood (J)
      J. Spores 3.3–4.3 μ broad, pleurocystidia absent or very rare, cheilocystidia apparently absent, pileus and volva avellaneous.....5. VOLVARIELLA JAMAICENSIS
      J. Spores 4.4–6.9 μ broad, pleurocystidia common to very abundant (K)
        K. Pileus and volva dark fuliginous.....6. VOLVARIELLA BAKERI
        K. Pileus white or yellow, volva white (with sordid yellowish to isabelline areolae in age in *V. bombycina*) (L)

L. Pileus dry, silky fibrillose and in age more or less squamulose, with margin not striate (M)
M. Pileus white, yellowish or sordid on disc in age.....7a. VOLVARIELLA BOMBYCINA var. BOMBYCINA
M. Pileus yellow.....7b. VOLVARIELLA BOMBYCINA var. FLAVICEPS
L. Pileus viscid, glabrous, with the margin finely striate.....8. VOLVARIELLA PECKII
I. Pileus < 5 cm broad or, if broader, then habitat not on wood (N)
N. Habitat on wood, soil, or dung (O)
O. Pileus 5–10 cm broad, prominently fibrillose with dark, appressed fibrils, dark brown to blackish brown (P)
P. Pileus streaked with blackish fibrils, often radially rimose; spores averaging 8.4–9.1 × 5.4–5.8 μ; cheilocystidia often more than 50 μ long.....9. VOLVARIELLA VOLVACEA
P. Pileus minutely fibrillose, spores averaging 7–7.6 × 4.6–4.8 μ, cheilocystidia not exceeding 45 μ in length.....10. VOLVARIELLA CUBENSIS
O. Pileus 0.5–6 cm broad, white, vinaceous brown, or brownish gray to brownish black (Q)
Q. Spores 9–12 μ long..19. *Volvaria avellanea* and 21. *Volvaria concinna*
Q. Spores < 9 μ long (R)
R. Pileus not white (at least not on disc), volva usually colored (S)
S. Pileus darkly colored (*i.e.*, not white) overall (see also 20. *Volvaria cinerea* and 22. *Volvaria submyochroa*) (T)
T. Volva not covered by long, white, mycelioid hairs (U)
U. Spores 4.1–6 μ broad.....11. VOLVARIELLA TAYLORI
U. Spores 3–3.7 μ broad.....12. VOLVARIELLA LEPIOTOSPORA
T. Volva with long, white, mycelioid hairs.....13. VOLVARIELLA VILLOSAVOLVA
S. Pileus white except for disc (V)
V. Spores 7–8.6 × 4.4–5.6 μ.....14. VOLVARIELLA ALACHUANA
V. Spores 4.7–7 × 3.1–3.9 μ.....15. VOLVARIELLA SMITHII
R. Pileus and volva white, at times becoming grayish in age (W)
W. Pileus 0.5–3 cm broad, not squamulose, becoming striate on the margin; stipe 1–5 cm long, glabrous..16. VOLVARIELLA PUSILLA
W. Pileus 2–5 cm broad, becoming squamulose, with the margin not striate; stipe 2–8 cm long, densely pubescent to villose, glabrescent below.....17. VOLVARIELLA HYPOPITHYS
N. Habitat on agarics.....18. VOLVARIELLA SURRECTA

ACCEPTED SPECIES

1. **Volvariella canalipes** (Murr.) comb. nov.
Volvariopsis canalipes Murr., Mycologia **30**: 368. 1938. (!)
Volvaria canalipes (Murr.) Murr., Mycologia **30**: 371. 1938.

FIG. 1

"Pileus convex, solitary, about 6–8 cm. broad; surface smooth, white, dry, glabrous, decorated with large fragments of the volva, margin not

striate; context white, with pleasant odor; lamellae free, crowded, ventricose, white to pink; . . . stipe white, tapering upward, very short, less than 4 cm., distinctly and closely furrowed for its entire length; volva large, white, cup-like, shallow, with ragged edges." (Murrill, **1938**).

Spores 15.7–22.9 (av. 18) × 8.7–11.4 (av. 10) μ, oval to ovoid, occasionally obovoid, Q = 1.64–2.00 (av. 1.79). Basidia 30–46 × 12–18 μ, clavate, 4-spored. Pleurocystidia 38–65 × 11–22 μ, fusoid to fusoid ventricose, with the apex either acute or obtuse and the neck sometimes narrowed to a slender projection 5–11 μ long, common. Cheilocystidia 30–68 × 12–29 μ, fusoid to fusoid-ventricose and then at times with the neck curved or narrowed into a slender projection, clavate, or obtusely lanceoloid, common. Subhymenium of cells 8–23 × 4–13 μ. Lamella trama of hyphae 4–25 μ in diameter. Pileus trama of hyphae 5–21 μ in diameter. Pileus cuticle non-gelatinous, of hyphae 8–36 μ in diameter. Clamp connections absent.

HABIT AND HABITAT. Solitary on sandy soil.

SPECIMEN EXAMINED. FLORIDA: Murrill F9975, Green Cove Springs, Clay Co., March 3, 1926 (FLAS, HOLOTYPE).

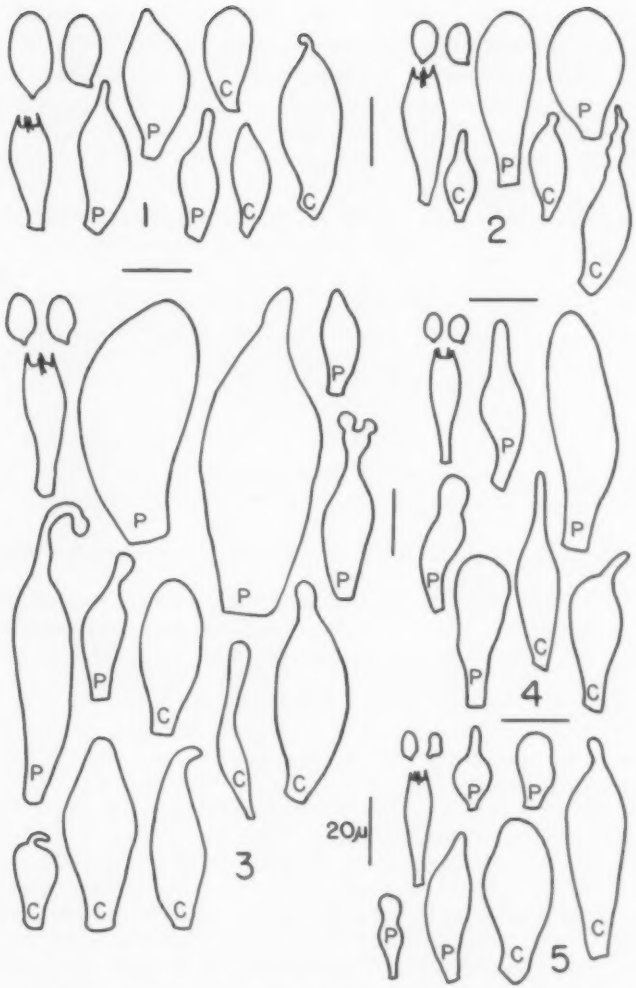
Murrill (**1938**) described the grooves on the stipe as being "deep, straight, close, exactly parallel, with sharp edges, reminding one of those found in some boletes, but without the cross-connections." They are prominent even in the dried specimen.

2. **Volvariella earlei** (Murr.) comb. nov.
Volvariopsis earlei Murr., Mycologia **3**: 282. 1911. (!)
Volvaria earlei (Murr.) Murr., Mycologia **4**: 332. 1912.

FIG. 2

Pileus 3–5 cm broad, convex, expanding to plano-convex, glabrous, bearing patches of the universal veil, dry or slightly viscid, short-striate on the margin or not, white to pale gray, discoloring in age. Lamellae close to crowded, moderately broad, ventricose, free, white, becoming flesh-pink. Stipe 4–10 cm × 5–8 mm, tapering upward from a slightly enlarged base, solid, glabrous, white to pale gray. Volva 5–10 mm high, vaginate, white to pale gray.

Spores 11.2–15.4 (av. 12.9–13.6) × 6.9–9.7 (av. 7.9–8.6) μ, usually ovoid, occasionally oval or obovoid, Q = 1.37–1.86 (av. 1.51–1.67). Basidia 27–47 × 10–17 μ, clavate, usually 4-, rarely 2-spored. Pleurocystidia 32–60 × 15–33 μ, clavate to obovoid, rare. Cheilocystidia 25–79 × 7–31 μ, fusoid, fusoid-ventricose, lanceoloid, clavate, or subglobose, with the apex at times bearing a small knob or a slender projection up to 47 μ long, common. Subhymenium of cells 8–32 × 4–18 μ. Lamella and pileus trama of hyphae 2–29 μ in diameter. Pileus cuticle gelatinous



FIGS. 1–5. *Volvariella*. Spores, basidia, pleurocystidia (P), and cheilocystidia (C). 1. *V. canalipes*. 2. *V. earlei*. 3. *V. speciosa* var. *speciosa*. 4. *V. bakeri*. 5. *V. bombycina* var. *flaviceps*.

or non-gelatinous, of hyphae 2–22 μ in diameter. Clamp connections absent.

HABIT AND HABITAT. Solitary to gregarious on soil.

SPECIMENS EXAMINED. CUBA: Earle 45, Santiago de las Vegas, June 4, 1904 (NY, HOLOTYPE; ISOTYPE at CUP-A). Earle 103, Santiago de las Vegas, June 18, 1904 (NY, PARATYPE). Van Herman (Earle 168), Santiago de las Vegas, Sept. 8, 1904 (NY, PARATYPE). 3 additional collections (NY).

ILLUSTRATIONS. Coker (**1947**), pl. 29 (photograph of carpophore); pl. 32, figs. 3–6 (drawings of carpophore, spores, basidium, and cystidium).

The description of macroscopic characteristics is adapted from the original description of the species and from notes accompanying herbarium specimens. A gelatinous cuticle could not be demonstrated on all the specimens examined, but evidently it is typically present in *V. earlei*.

3. **Volvariella alabamensis** (Murr.) comb. nov.

Volvariopsis alabamensis Murr., N. Amer. Flora **10**: 144. 1917. (!)

Volvaria alabamensis (Murr.) Murr., Mycologia **9**: 180. 1917.

"Pileus thick, firm, convex, not umbonate, solitary, 6–7 cm. broad; surface smooth, dry, glabrous with a silky sheen, unifórmly dark-brown, margin entire, concolorous, not striate; lamellae free, broad, ventricose, crowded, white to salmon-colored, somewhat undulate on the edges; . . . stipe short, enlarged below, smooth, glabrous, white, solid, 4–5 cm. long, 5–10 mm. thick; volva white, somewhat fibrillose, ample, closely adhering below, with free, lacerate margin, a portion being carried up on top of the pileus." (Murrill, **1917**).

Spores 14.7–20.3 (av. 17.5–17.6) × 8.6–10.7 (av. 9.3–9.6) μ, usually ovoid or oval, occasionally obovoid, Q = 1.67–2.09 (av. 1.84–1.88). Basidia 26–41 × 11–16 μ, clavate, usually 4-, rarely 2-spored. Pleurocystidia 44–65 × 13–21 μ, fusoid-ventricose, rare. Cheilocystidia apparently absent. Lamella trama of hyphae 4–32 μ in diameter. Pileus trama of hyphae 2–22 μ in diameter. Pileus cuticle non-gelatinous, of hyphae 4–33 μ in diameter. Clamp connections absent.

HABIT AND HABITAT. Solitary on soil.

SPECIMEN EXAMINED. ALABAMA: Earle, Auburn, Lee Co., March 10, 1898 (NY, HOLOTYPE; portion of HOLOTYPE at CUP-A).

The type specimen at NY has been almost completely destroyed by insects; only spores remain in the debris. The CUP-A material consists of a small radial portion of a pileus in poor condition. Pleuro-

cystidia did not revive well, and they are probably more abundant and more diverse in size and shape than is indicated in the above description, for which observations on only three cystidia were made. Cheilocystidia may also have been present but too badly collapsed to be identified.

V. alabamensis is close to *V. speciosa* and differs in its dark brown pileus which is not viscid, shorter and more narrow stipe, and perhaps cystidium characteristics. Murrill (**1938**) considered *V. alabamensis* to be the nearest relative of *Volvariopsis floridana* Murr., which is here listed as a synonym of *Volvariella speciosa.*

4a. VOLVARIELLA SPECIOSA (Fr. ex Fr.) Sing., Lilloa **22**: 401. [1951.]
var. SPECIOSA

[*Amanita speciosa* Fr., Obs. Myc. **2**: 1. 1818.]
Agaricus speciosus (Fr.) ex Fr., Syst. Myc. **1**: 278. 1821.
Agaricus (*Volvaria*) *emendatior* Berk. & Curt., Ann. Mag. Nat. Hist. III. **4**: 288. 1859. (!)
Volvaria speciosa (Fr. ex Fr.) Kummer, Führ. Pilzk. 99. 1871.
Volvaria emendatior (Berk. & Curt.) Lloyd, Comp. Volvae 12. 1898.
Volvariopsis speciosa (Fr. ex Fr.) Murr., N. Amer. Flora **10**: 143. 1917.
Volvariopsis emendatior (Berk. & Curt.) Murr., N. Amer. Flora **10**: 143. 1917.
Volvariopsis floridana Murr., Mycologia **30**: 368. 1938. (!)
Volvaria floridana (Murr.) Murr., Mycologia **30**: 371. 1938.

FIG. 3

Pileus 5–15 cm broad, ovoid to globose when young, expanding to convex or plane, more or less umbonate, viscid, glabrous, at times bearing patches of the universal veil, with the margin not striate or only slightly so, white to light avellaneous or light brownish gray, often darker on the disc; flesh moderately thick in center, thin towards the margin, soft, white, with unpleasant odor and taste. Lamellae close to crowded, broad, ventricose, free, with uneven to erose edges, white, becoming deep flesh color. Stipe 9–20 × 0.8–2 cm, equal or enlarging to base, terete, solid, glabrous or villose and glabrescent, with the base villose to tomentose and at times bulbous and up to 3 cm thick, white to cream. Volva rather shallow, with the margin free and nearly even, lacerate, or lobed, white to light gray.

Spores 11.7–20.9 (av. 13.4–18.1) × 7.2–12.4 (av. 8.3–10.3) μ, oval to ovoid, occasionally obovoid, Q = 1.35–2.04 (av. 1.53–1.84). Basidia 30–66 × 11–18 μ, clavate, 4-, rarely 2- or 3-spored. Pleurocystidia 30–118 × 10–69 μ, subcylindrical to fusoid and then with or without a

knob at the apex, fusoid-ventricose (and then at times with the neck enlarged at the apex, constricted at the base, rarely branched, slender and curved, or short and passing abruptly into the body of the cystidium), lanceoloid to oblanceoloid and then at times bearing a nipple or knob at the apex, clavate and then often with a knob or slender projection up to 25 μ long arising from the apex, ovoid, or obovoid, common. Cheilocystidia 23–108 × 8–42 μ, fusoid, with or without a projection up to 50 μ long and 8 μ wide which may be variously swollen and contorted along its length and rarely branched, fusoid-ventricose and then at times with the neck enlarged at the apex or slender and passing abruptly into the body of the cystidium, obtusely lanceoloid with or without a knob or nipple at the apex, clavate and then at times with a slender projection up to 25 μ long arising from the apex, or ovoid, abundant. Subhymenium of cells 5–38 × 2–26 μ. Lamella trama of hyphae 2–53 μ in diameter. Pileus trama of hyphae 2–58 μ in diameter. Pileus cuticle of gelatinized hyphae, up to 260 μ thick. Clamp connections absent.

HABIT AND HABITAT. Solitary to gregarious on dung or rich soil in lawns, gardens, fields, woods, and greenhouses.

SPECIMENS EXAMINED. ONTARIO: Dearness, Byron, Dec. 27, 1927 (NY). 2 additional collections (NY, DAOM). MANITOBA: Bisby, Matlock, July 17, 1927 (DAOM F6929). BRITISH COLUMBIA: Waugh, Burnaby Lake, June 9, 1954 (DAOM 44796). YUKON: Billard & Calder, Dawson, July 14, 1949 (DAOM 21732). NEW ENGLAND: Sprague (Curtis 5074) (K, LECTOTYPE of *Agaricus emendatior* Berk. & Curt.). NEW YORK: Jackson, Ithaca, Tompkins Co., Oct. 11, 1904 (CUP-A 18602). 2 additional collections (NYS). DISTRICT OF COLUMBIA: Braendle (Lloyd 44800), Washington (BPI). 1 additional collection (NYS). SOUTH CAROLINA: Curtis 2546 (K, SYNTYPE of *Agaricus emendatior* Berk. & Curt.). ALABAMA: Jones, Livingston, Sumter Co., Jan. 25, 1898 (BPI). FLORIDA: Murrill F16047, Gainesville, Alachua Co., Jan. 13, 1938 (FLAS, PARATYPE of *Volvariopsis floridana* Murr.). Murrill & Steckel F16046, Gainesville, Alachua Co., Jan. 17–21, 1938 (FLAS, HOLOTYPE of *Volvariopsis floridana* Murr.). Murrill F21513, Gainesville, Alachua Co., Feb. 14, 1941 (FLAS). Singer F1791, Gainesville, Alachua Co., Spring, 1943 (F). 6 additional collections (BPI, F, FLAS, NY). MICHIGAN: Lange 1423, Sleeping Bear Point, Leelanau Co., July 24–25, 1947 (MICH). Kauffman, Ann Arbor, Washtenaw Co., July 8, 1905 (MICH). 7 additional collections (CUP-A, MICH). INDIANA: Cole, West Lafayette, Tippecanoe Co., May 30, 1907 (CUP-A 21843). ILLINOIS: E. T. & S. A. Harper, Port Byron, Rock Island Co., June, 1900 (F1124782). 1 additional collection (F). WISCONSIN: Brown (NYS). SOUTH DA-

KOTA: Williams, Brookings, Brookings Co., June, 1896 (BPI). KANSAS: Rogerson 3728, Manhattan, Riley Co., June 11, 1954 (RLS). COLORADO: Sterling, Denver, Adams Co., June, 1907 (NYS). MONTANA: Kauffman, Echo Lake, Flathead Co., June 29, 1928 (MICH). 1 additional collection (MICH). WASHINGTON: Smith 48750, Tacoma, Pierce Co., Oct. 9, 1954 (MICH). OREGON: Zeller, Corvallis, Benton Co., June, 1927 (NY). 1 additional collection (NYS). CALIFORNIA: Krieger, Chico, Butte Co., May 18, 1913 (NYS). Yates 80, Berkeley, Alameda Co., Dec. 24, 1913 (NY). Brandenburg (Morse 266e), Mohave Desert, Apr. 23, 1940 (BPI). McClatchie 39, Pasadena, Los Angeles Co., 1893 (NY). 15 additional collections (BPI, DAOM, MICH, NY, NYS).

ILLUSTRATIONS. Coker (**1947**), pl. 30 (photographs of carpophores and pileus); pl. 32, fig. 2 (drawing of spores), figs. 7–8 (drawings of spores of *Volvariopsis floridana* Murr.).

Descriptions of the macroscopic characteristics of *Agaricus emendatior* Berk. & Curt. provide nothing that would exclude this species from synonymy under *Volvariella speciosa*. Those microscopic characteristics which could be checked on the lectotype and syntype specimens from Kew are in agreement with those of *V. speciosa*. Although there is no mention of viscidity in the original description (the species was described from dried material sent to Berkeley by Curtis), both of the Kew specimens show a thin, but distinct layer of gelatinized hyphae on the surface of the pileus.

Volvariopsis floridana Murr. is also treated as a synonym of *Volvariella speciosa* for lack of distinctive characteristics to distinguish it. Coker (**1947**) considered *floridana* to differ from *speciosa* in the glabrous stipe and less viscid pileus which bears patches of the universal veil, and Murrill (**1951**), in the darker color (avellaneous) and less viscid pileus. In the collections of *Volvariella speciosa* examined the vesture of the stipe was variable, and glabrous stipes were not uncommon. The thickness of the layer of gelatinized hyphae covering the pileus had a large range. Veil patches on the pileus were most numerous in the type specimens of *Volvariopsis floridana* but were found occasionally in *Volvariella speciosa* as well, and variations in color are common in the two varieties of *V. speciosa* here recognized. Microscopic characteristics provide nothing upon which to recognize *floridana*. Size and shape of the spores, basidia, and cystidia are all within the range of *V. speciosa* although the cheilocystidia of Murrill & Steckel F16046, Murrill F21513, and Singer F1791 (all from Florida) are more commonly of bizarre shapes than of other specimens of *V. speciosa* studied.

See also the notes following *V. speciosa* var. *gloiocephala*.

4b. VOLVARIELLA SPECIOSA (Fr. ex Fr.) Sing. var. GLOIOCEPHALA (DC. ex Fr.) Sing., Lilloa **22**: 401. [1951.]

[*Agaricus gloiocephalus* DC., Flore Franç. **6**: 52. 1815.]

Agaricus gloiocephalus DC. ex Fr., Syst. Myc. **1**: 278. 1821. (ut *glojocephalus*)

Volvaria gloiocephala (DC. ex Fr.) Gill., Les Champ. 387. 1878.

Volvaria speciosa (Fr. ex Fr.) Kummer var. *gloiocephala* (DC. ex Fr.) Heim, Rev. Mycol., Paris **1** (Suppl.): 89. 1936.

Pileus 5–10 cm broad, ovoid when young, expanding through campanulate to convex and plane, more or less umbonate, viscid, glabrous, at times bearing patches of the universal veil, with the margin striate, dull white or fulvous to pearl gray or fuliginous; flesh thin, white. Lamellae close to crowded, broad in front, narrowed behind, free, with the edges fimbriate, white, then flesh color. Stipe 8–21 × 0.5–2 cm, enlarging to base, terete, solid, glabrous or more or less villose at base, white. Volva with the margin even, lacerate, or lobed, white.

Spores 11.2–17.6 (av. 12.2–16.1) × 6.7–10.2 (av. 7.7–8.9) μ, ovoid to oval, occasionally obovoid, Q = 1.48–2.00 (av. 1.59–1.81). Basidia 31–52 × 11–15 μ, clavate, 4-spored. Pleurocystidia 40–92 × 14–37 μ, with shapes and frequency as in var. *speciosa*. Cheilocystidia 38–70 × 10–33 μ, with shapes and frequency as in var. *speciosa*. Other microscopic characteristics as in var. *speciosa*.

HABIT AND HABITAT. Solitary to gregarious on rich soil in woods.

SPECIMENS EXAMINED. NEW YORK: Bradfield, Ithaca, Tompkins Co., Aug. 30, 1902 (CUP-A 13180). Edgerton, Taughannock Falls, Tompkins Co., July 28, 1906 (CUP-A 19926). IOWA: Hess & Vandivert 8, Ames, Story Co., May 25, 1899 (NYS). KANSAS: Bartholomew, Stockton, Rooks Co., July 5, 1899 (BPI). 1 additional collection (BPI). CALIFORNIA: Smith 329, Berkeley, Alameda Co., Feb. 20, 1906 (CUP-A). 3 additional collections (NY, NYS).

The taxon *gloiocephala* has often been recognized as a species. Kauffman (**1918**) wrote of *Volvaria gloiocephala* that "except for the darker colors, smaller spores, striations on the pileus, and lack of cystidia, this seems close to [*V. speciosa*] and might perhaps be considered a variety of it." Most authors, however, separate the two only on the basis of color and the presence or absence of striations.

No break in either length or width of spores was found in the specimens examined. Two of those with larger spores, CUP-A 18602 and CUP-A 21843 (see under var. *speciosa*) are accompanied by notes which describe the pileus as striate. No specimens lacked cystidia; both pleuro- and cheilocystidia were common in all lamella sections.

The color distinction has usually been drawn on the basis of white (*speciosa*) vs. brown or gray (*gloiocephala*) (Murrill, 1917; Kauffman, 1918; Coker, 1947), but the colors are variable, intergrade, and again are not correlated with presence or absence of striations, *e.g.*, in CUP-A 19926 and CUP-A 13180 (under var. *gloiocephala*) and in CUP-A 18602 and CUP-A 21843 (under var. *speciosa*).

It seems, therefore, that if *gloiocephala* and *speciosa* are recognized as species, the separation must be made on the basis of the striation characteristic, which is also not absolute as is pointed out in the descriptions above. Although specimens have been placed in either var. *speciosa* or var. *gloiocephala* on the basis of the separation given in the key, this placement has frankly been arbitrary in some cases, for the two varieties intergrade to a great extent.

5. **Volvariella jamaicensis** (Murr.) comb. nov.
Volvariopsis jamaicensis Murr., Mycologia **3**: 281. 1911. (!)
Volvaria jamaicensis (Murr.) Murr., Mycologia **4**: 332. 1912.

"Pileus thin, convex to nearly plane, gregarious, 5 cm. broad; surface ashy-white, avellaneous at the center, radiate-striate, slightly granular, margin thin, entire; lamellae free, close, narrow, white to salmon-colored; . . . stipe curved, slightly tapering upward, glabrous, whitish, hollow, with a tough rind, 5 cm. long, 3–5 mm. thick; volva rather delicate, narrow, avellaneous, 1–2 cm. long." (Murrill, **1911**).

Spores 6.2–8 (av. 7) × 3.3–4.3 (av. 3.9) μ, oval to ovoid, occasionally obovoid, Q = 1.54–2.08 (av. 1.81). Basidia 18–30 × 6–9 μ, clavate, 4-spored. Pleurocystidia either absent or very rare. Cheilocystidia apparently absent. Subhymenium of cells 12–32 × 11–23 μ. Lamella trama of hyphae 2–11 μ in diameter. Pileus trama of hyphae 2–23 μ in diameter. Pileus cuticle non-gelatinous, of hyphae 2–21 μ in diameter. Clamp connections absent.

HABIT AND HABITAT. Gregarious on dead wood.

SPECIMEN EXAMINED. JAMAICA: W. A. & Edna Murrill 150, Moore Town, Dec. 16, 1908 (NY, HOLOTYPE; ISOTYPE at CUP-A).

6. **Volvariella bakeri** (Murr.) comb. nov.
Volvariopsis bakeri Murr., Mycologia **3**: 281. 1911. (!)
Volvaria bakeri (Murr.) Murr., Mycologia **4**: 332. 1912.

FIG. 4

"Pileus fleshy, ovoid to convex, densely gregarious, reaching a breadth of 10 cm.; surface dark-fuliginous, becoming much lighter with

age, appressed fibrillose with the cracking of the cuticle, not striate; context white, with mild taste and no appreciable odor; lamellae free, crowded, not very broad, white, becoming pink; . . . stipe tapering upward, white, glabrous, solid, 8–9 cm. long, 1–1.5 cm. thick; volva free, open, dark-fuliginous, 3–4 cm. long, 2–3 cm. broad." (Murrill, **1911**).

Spores 6.9–9.3 (av. 7.9–8.2) × 4.6–6.9 (av. 5.1–5.7) μ, usually ovoid, occasionally oval or obovoid, $Q = 1.32–1.91$ (av. 1.44–1.55). Basidia (21–)27–35 × 7–11 μ, clavate, usually 4-, rarely 2-spored. Pleurocystidia 42–71 × 11–31 μ, fusoid, fusoid-ventricose and then at times with the neck elongate, clavate, or dumbbell-shaped, abundant. Cheilocystidia 27–79 × 11–27 μ, fusoid-ventricose and then at times with an elongate or branched neck, or clavate and then at times with a small knob or projection up to 15 μ long arising from the apex, abundant. Subhymenium of cells 10–23 × 8–15 μ. Lamella trama of hyphae 2–19 μ in diameter. Pileus trama of hyphae 3–28 μ in diameter. Pileus cuticle non-gelatinous, of hyphae 4–28 μ in diameter. Clamp connections absent.

HABIT AND HABITAT. Solitary to gregarious on wood.

SPECIMENS EXAMINED. CUBA: Baker (Earle 521), Santiago de las Vegas, May 19, 1906 (NY, HOLOTYPE; ISOTYPE at CUP-A). PUERTO RICO: Cooke (Lloyd 56095), July, 1925 (BPI).

V. bakeri is obviously very close to *V. bombycina*. It might better be considered as only a dark-colored form of the latter species, and further collections could prove it to be just that. However, it evidently does differ also in not having the pileus silky fibrillose and in age more or less squamulose and in lacking the large cystidia of *V. bombycina*.

7a. VOLVARIELLA BOMBYCINA (Schaeff. ex Fr.) Sing., Lillio **22**: 401. [1951.] var. BOMBYCINA

[*Agaricus bombycinus* Schaeff., Fung. Bav. Pal. Rat. Ic. **4**: 42 (index). 1774.]

Agaricus bombycinus Schaeff. ex Fr., Syst. Myc. **1**: 277. 1821.

Volvaria bombycina (Schaeff. ex Fr.) Kummer, Führ. Pilzk. 99. 1871.

Volvariopsis bombycina (Schaeff. ex Fr.) Murr., Mycologia **3**: 281. 1911.

Volvariopsis earleae Murr., N. Amer. Flora **10**: 142. 1917. (!)

Volvaria earleae (Murr.) Murr., Mycologia **9**: 180. 1917.

FIG. 6

Pileus 5–20 cm broad, globose to ovoid when young, expanding to campanulate or convex and perhaps finally to nearly plane, dry, silky

fibrillose, in age somewhat squamulose, with the margin more or less fimbriate and not striate, white, in age yellowish or sordid on the disc; flesh thin, soft, white, with mild taste and slight odor. Lamellae crowded, broad, ventricose, free, with erose edges, white, becoming flesh color. Stipe 6–20 × 1–2 cm, enlarging to the base which may be bulbous, terete, solid, glabrous, white. Volva ample, thick, with the margin lobed or nearly even and the surface areolate, whitish, in age sordid yellowish or isabelline on the areolae.

Spores 6.6–10.4 (av. 7.3–9.4) × 4.4–6.7 (av. 4.8–5.7) μ, usually ovoid, occasionally oval, oblong, or obovoid, Q = 1.25–1.85 (av. 1.42–1.66). Basidia 19–43 × 6–11 μ, clavate, 4-spored. Pleurocystidia 26–122 × 8–57 μ, usually fusoid-ventricose and then at times with the neck enlarged at the apex, but also fusoid, obtusely lanceoloid, or clavate, rarely ovoid, obovoid, or dumbbell-shaped, common to very abundant. Cheilocystidia 26–144 × 8–46 μ, fusoid, fusoid-ventricose and then at times with the neck narrow or elongate or slightly constricted at the base or curved or variously swollen, obtusely lanceoloid to clavate and then at times bearing a knob or a slender projection up to 20 μ long at the apex, or ovoid, very abundant. Subhymenium of cells 4–31 × 4–21 μ. Lamella trama of hyphae 2–29 μ in diameter. Pileus trama of hyphae 2–36 μ in diameter. Pileus cuticle non-gelatinous, of hyphae 4–35 μ in diameter. Clamp connections absent.

HABIT AND HABITAT. Solitary to gregarious on wood (sugar maple, red maple, silver maple, magnolia, mango, beech, oak, American elm).

SPECIMENS EXAMINED. QUEBEC: Güssow, Burnet, July 24, 1938 (DAOM 8842). ONTARIO: Archibald, Ottawa, July 11, 1930 (DAOM F7866). 11 additional collections (DAOM). CONNECTICUT: Underwood & Earle 1201, Redding, Fairfield Co., Aug. 23, 1902 (NY). NEW YORK: Burnham, n. of Hudson Falls, Washington Co., Aug. 30, 1914 (CUP 22902). Thomas, Long Island, July 8, 1913 (NY). Johnson, Utica, Oneida Co., Aug. (NYS). Murrill, Ithaca, Tompkins Co., Aug. 20, 1898 (CUP-A 3096). Deegan 53, Tonawanda, Erie Co., 1949 (MICH). 16 additional collections (CUP, CUP-A, NYS). NEW JERSEY: Anderson, Lambertville, Hunterdon Co., July, 1905 (NYS). 1 additional collection (MICH). PENNSYLVANIA: Martin, West Chester, Chester Co., Aug., 1879 (NY). MARYLAND: May & Hartley, Beltsville, Montgomery Co., Sept. 14, 1950 (BPI). DISTRICT OF COLUMBIA: Lewton, Washington, June, 1905 (BPI). 3 additional collections (BPI). FLORIDA: West, Gainesville, Alachua Co., June 11, 1940 (NY). MISSISSIPPI: E. Earle 60, Biloxi, Harrison Co., Sept. 2, 1904 (NY, HOLOTYPE of *Volvariopsis earleae* Murr.). OHIO: Walters, Cleveland, Cuyahoga Co., Aug. 15, 1948 (MICH). MICHIGAN: Smith

49929, Tahquamenon Falls St. Pk., Luce Co., Aug. 5, 1955 (MICH). Smith 25799, Wolf Bog, Cheboygan Co., July 18, 1947 (MICH). Lee 642, Ann Arbor, Washtenaw Co., Aug. 9, 1907 (MICH). 2 additional collections (MICH). INDIANA: Edgecombe, Tremont, Porter Co., Oct., 1939 (F1207600). ILLINOIS: Edgecombe, Evanston, Cook Co., Sept. 10, 1938 (F1202736). E. T. & S. A. Harper 88, Geneseo, Henry Co., Aug., 1898 (F1124781). 4 additional collections (F). IOWA: E. T. & S. A. Harper, Sabula, Jackson Co., Aug., 1903 (F1124775). 1 additional collection (NYS). KANSAS: Rogerson 3730, Mildred, Allen Co., June 9, 1955 (KSC). WASHINGTON: Weir 15466, Spokane, Spokane Co., Sept., 1919 (BPI). CUBA: Earle 564, Herradivia, June 27, 1907 (NY). 1 additional collection (NY).

ILLUSTRATIONS. Atkinson (**1900**), fig. 134 (photograph of carpophore). Coker (**1947**), pl. 28 (photograph of carpophores); pl. 31, fig. 1 (drawing of spores). Hard (**1908**), pl. 29, figs. 191–193 (photographs of carpophores). Kauffmann (**1918**), pl. 100 (photograph of carpophores). Lloyd (**1898**), fig. 8 (photograph of carpophore). McIlvaine (**1900**), pl. 59 (photograph of carpophores). A. H. Smith (**1945**), p. 441 (photograph of carpophore).

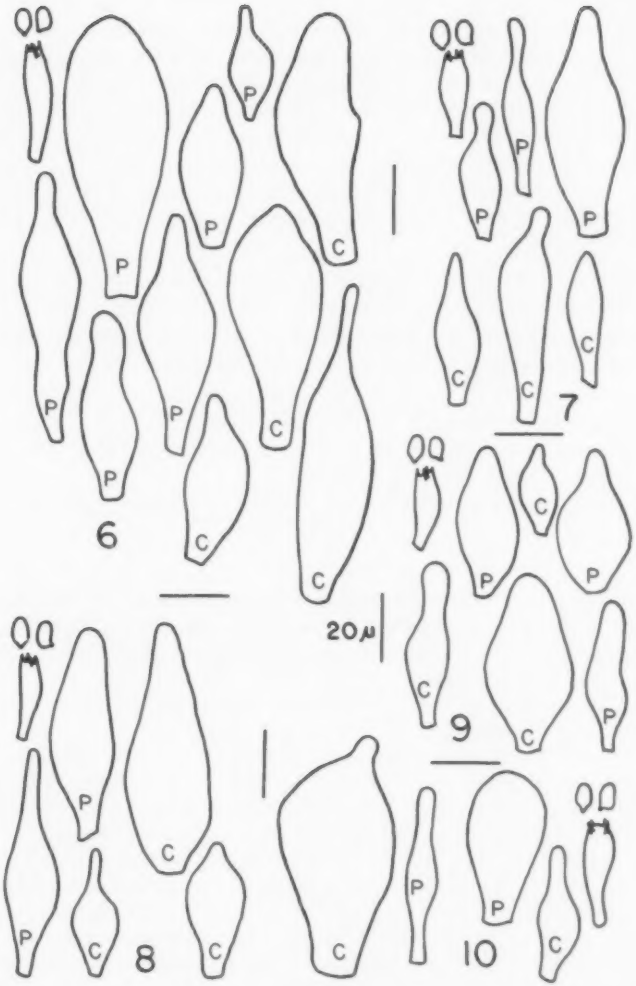
The type specimen of *Volvariopsis earleae* Murr. has been destroyed by insects; however, a radial section of a pileus from this specimen is at CUP-A. On the basis of the published description, the notes and sketch accompanying the specimen, and the data on microscopic characteristics obtained from the CUP-A material, the binomial *Volvariopsis earleae* appears to be based on an unexpanded carpophore of *Volvariella bombycina*. Nothing now known excludes this possibility.

7b. VOLVARIELLA BOMBYCINA (Schaeff. ex Fr.) Sing. var. **flaviceps** (Murr.) stat. nov.

Volvaria flaviceps Murr., Mycologia **41**: 490. 1949. (!)

FIG. 5

"Pileus campanulate, solitary, 3.5 cm. broad; surface dry, smooth, fibrillose, uniformly bright-flavous, margin fimbriate, projecting 2 mm.; context very thin, white, unchanging, with a peculiar sickening odor during drying; lamellae free, broad, wide and rounded at the margin, narrow behind, crowded, very thin, entire, white, unchanging, sterile; stipe smooth, white, glabrous, curved, enlarging below, 4 × 0.6–1 cm. above the volva, which is 4 cm. high and 2.5 cm. broad at the top, tapering to one centimeter at the base, dry, dirty-white with large, flat, pale-umbrinous scales, their acute tips pointed upward." (Murrill, **1949**).



FIGS. 6–10. *Volvariella*. Spores, basidia, pleurocystidia (P), and cheilocystidia (C). 6. *V. bombycina* var. *bombycina*. 7. *V. peckii*. 8. *V. volvacea*. 9. *V. cubensis*. 10. *V. taylori*.

Spores 7.2–10 (av. 7.8–8.6) × 4.7–6.3 (av. 5.2–5.6) μ, ovoid to oval, Q = 1.23–1.82 (av. 1.41–1.53). Basidia 28–46 × 8–10 μ, clavate, 4-spored. Pleurocystidia 21–93 × 8–33 μ, fusoid, fusoid-ventricose and then at times with the neck short or knob-like, dumbbell-shaped, lanceoloid, ovoid, or clavate, abundant. Cheilocystidia 35–78 × 12–26 μ, fusoid, fusoid-ventricose, obtusely lanceoloid, or clavate and then at times with the apex acute or bearing a projection up to 10 μ long, abundant. Subhymenium of cells 4–21 × 4–18 μ. Lamella trama of hyphae 2–19 μ in diameter. Pileus trama of hyphae 2–22 μ in diameter. Pileus cuticle non-gelatinous, of hyphae 7–33 μ in diameter. Clamp connections absent.

HABIT AND HABITAT. Solitary to gregarious on wood (magnolia).

SPECIMENS EXAMINED. FLORIDA: Murrill F22458, Mary's Sink, Gainesville, Alachua Co., July 11, 1948 (FLAS, HOLOTYPE). Murrill F44309, Gainesville, Alachua Co., Oct. 1, 1949 (FLAS).

8. **Volvariella peckii** (Atk. in Pk.) comb. nov.

Volvaria peckii Atk. in Pk., 48th Rep. N. Y. St. Mus. 1894: 11. 1896. (!)

Volvariopsis peckii (Atk. in Pk.) Murr., N. Amer. Flora **10**: 142. 1917.

FIG. 7

"Pileus thin, convex, glabrous, viscid, finely striate on the margin, whitish; lamellae rather close, thin, pale flesh color; stem slightly tapering upward, glabrous, solid, whitish, with a loose, well-developed membranous volva at the base; . . .

"Pileus about 3 in. broad; stem 3 to 3.5 in. long, 3–4 lines thick." (Atkinson in Peck, **1896**).

Spores 7.7–10.7 (av. 8.9) × 4.9–6.6 (av. 5.6) μ, ovoid to oval, occasionally oblong or obovoid, Q = 1.33–1.79 (av. 1.59). Basidia 24–31 × 8–11 μ, clavate, 4-, rarely 2-spored. Pleurocystidia 41–69 × 10–26 μ, fusoid or more commonly fusoid-ventricose and then at times with the apex slightly enlarged, common to abundant. Cheilocystidia 30–83 × 10–19 μ, usually fusoid-ventricose and then at times with the neck short or slender and strict or enlarged at the apex, occasionally fusoid or clavate, common. Subhymenium of cells 8–22 × 7–18 μ. Lamella trama of hyphae 5–22 μ in diameter. Pileus trama of hyphae 5–32 μ in diameter. Pileus cuticle non-gelatinous (?), of hyphae 5–28 μ in diameter. Clamp connections absent.

HABIT AND HABITAT. Solitary on rotted wood.

SPECIMEN EXAMINED. NEW YORK: Atkinson 249, Ithaca, Tompkins Co., Sept. 25, 1893 (CUP-A, HOLOTYPE).

Although Atkinson described the pileus as viscid, no cuticle of gelatinized hyphae such as is found in *Volvariella speciosa* could be demonstrated on the poorly preserved type specimen. *V. peckii* is evidently closest to *V. bombycina* among the North American species, but the glabrous, questionably viscid pileus with striate margin should readily distinguish it.

9. VOLVARIELLA VOLVACEA (Bull. ex Fr.) Sing., Lilloa **22**: 401. [1951.]
[*Agaricus volvaceus* Bull., Herb. Fr., pl. 262. 1780.]
[*Agaricus virgatus* Pers., Tent. Disp. Meth. Fung. 18. 1797.]
[*Amanita virgata* Pers., Tent. Disp. Meth. Fung. 66. 1797.]
Agaricus volvaceus Bull. ex Fr., Syst. Myc. **1**: 278. 1821.
Volvaria volvacea (Bull. ex Fr.) Kummer, Führ. Pilzk. 99. 1871.
Volvaria virgata (Pers.) ex Quél., Mém. Soc. Emul. Montbéliard II. **5**: 344. 1873.
Volvariopsis volvacea (Bull. ex Fr.) Murr., N. Amer. Flora **10**: 144. 1917.

FIG. 8

Pileus 5–10 cm broad, ovoid when young, expanding to campanulate or convex, at times subumbonate or depressed on the disc, dry, virgate with dark fibrils, often radially rimose, with the margin not striate, fuliginous to grayish brown, blackish brown on disc; flesh 3–5 mm thick on disc, tapering more or less evenly to margin of pileus, soft, thin, with mild taste and meaty-aromatic odor (Coker, **1947**), white, tinged with surface color beneath the cuticle or not. Lamellae close, broad, free, with fimbriate edges, white when young, becoming deep flesh-color. Stipe 4.5–14 cm × 3–20 mm, subequal above or enlarging slightly to subbulbous base, terete, stuffed, becoming hollow, innately fibrillose, off-white to dull brown. Volva large, with margin free and irregularly lobed, membranaceous, floccose, brownish.

Spores 6.9–10.4 (av. 8.4–9.1) × 4.6–7 (av. 5.4–5.8) μ, oval to ovoid, occasionally obovoid, Q = 1.30–1.87 (av. 1.46–1.65). Basidia 20–44 × 7–11 μ, clavate, 4-spored. Pleurocystidia 35–113 × 8–36 μ, subcylindrical, fusoid-ventricose and then at times with the neck very short or elongate and enlarged at the apex, occasionally dumbbell-shaped, lanceoloid, or clavate, rarely fusoid, common to abundant. Cheilocystidia 23–107 × 8–29 μ, usually fusoid-ventricose and then at times with the neck elongate or enlarged at the apex, clavate with the apex acute or obtuse and rarely with a small knob, or lanceoloid, abundant. Subhymenium of cells 7–29 × 5–18 μ. Lamella trama of hyphae 2–24 μ in diameter. Pileus trama of hyphae 2–33 μ in diameter. Pileus cuticle non-gelatinous, of hyphae 3–28 μ in diameter. Clamp connections absent.

HABIT AND HABITAT. Solitary to gregarious on soil or compost piles; according to Lloyd (**1898**) *et al.* often in hothouses and cellars.

SPECIMENS EXAMINED. ONTARIO: Groves, Ottawa, Aug. 31, 1949 (DAOM 21720). 3 additional collections (DAOM). MASSACHUSETTS: Morris, Waltham, Middlesex Co., 1902 (BPI). NEW YORK: Pivout, N. Y. Bot. Gard., Bronx Co., ?1949 (NY). OHIO: Lloyd 45238, Cincinnati, Hamilton Co., 1902 (BPI). MICHIGAN: Smith, Oak Park, Oakland Co., Aug. 6, 1942 (MICH). DISTRICT OF COLUMBIA: Charles, Washington, July 17, 1932 (BPI). FLORIDA: Murrill F15164, Gainesville, Alachua Co., Apr. 30, 1938 (NY). 2 additional collections (MICH, NY). BERMUDA: Seaver & Waterston (Fungi of Bermuda 477), Paget, Jan. 7, 1942 (NY). 1 additional collection (MICH).

ILLUSTRATIONS: Coker (**1947**), pl. 31 (drawing of carpophores). Lloyd (1898), fig. 9 (photograph of carpophore). McIlvaine (**1900**), pl. 60 (drawing of carpophores).

See the discussion under *Volvariella cubensis* and *V. taylori*.

10. **Volvariella cubensis** (Murr.) comb. nov.
Volvariopsis cubensis Murr., Mycologia **3**: 281. 1911. (!)
Volvaria cubensis (Murr.) Murr., Mycologia **4**: 332. 1912.

FIG. 9

"Pileus firm, fleshy, rather tough, irregularly expanded, obtuse, solitary, 7 cm. broad, with strong, unpleasant odor; surface dark smoky-brown, minutely fibrillose, not striate, the disk seal-brown and glabrous; lamellae free, distant, subcrowded, rather broad, subventricose, heterophyllous; . . . stipe subcylindric, slightly enlarged above and below, concolorous but paler, glabrous, solid, tough, apex pallid, 6–7 cm. long, 7 mm. thick; volva thick and fleshy, cup-shaped, distant, bifid, concolorous." (Murrill, **1911**).

Spores 6.2–8.6 (av. 7–7.6) × 4.3–5.3 (av. 4.6–4.8) μ, oval to ovoid, occasionally obovoid, Q = 1.35–1.88 (av. 1.46–1.67). Basidia 18–30 × 7–11 μ, clavate, 4-spored. Pleurocystidia 27–63 × 9–33 μ, fusoid, fusoid-ventricose and then at times with the apex enlarged or approaching dumbbell-shaped, obtusely lanceoloid, clavate, or ovoid, common. Cheilocystidia 14–42 × 10–27 μ, fusoid-ventricose and then at times with the neck elongate or enlarged at the apex, dumbbell-shaped, clavate and then at times with a knob at the apex, or occasionally ovoid or obovoid, locally abundant. Subhymenium of cells 5–29 × 4–21 μ. Lamella trama of hyphae 2–32 μ in diameter. Pileus trama of hyphae 4–33 μ in diameter. Pileus cuticle non-gelatinous, of hyphae 4–36 μ in diameter. Clamp connections absent.

HABIT AND HABITAT. Solitary on soil.

SPECIMENS EXAMINED. CUBA: Earle 17, Santiago de las Vegas, May 13, 1904 (NY, HOLOTYPE; ISOTYPE at CUP-A). Van Herman (Earle 180), Santiago de las Vegas, Sept. 11, 1904 (NY, PARATYPE). 2 additional collections (NY).

Volvariella cubensis and *V. volvacea* are quite similar, and here again studies of more specimens could reveal variation which would lead to the conclusion that they are conspecific. Statistical analyses of the differences in spore size between the two species show that these differences are highly significant.

11. VOLVARIELLA TAYLORI (Berk.) Sing., Lilloa **22**: 401. [1951.]
Agaricus (*Volvaria*) *taylori* Berk., Outl. Brit. Fungol. 140. 1860.
Volvaria taylori (Berk.) Gill., Les Champ. 386. 1878.
?*Volvariopsis fimetaria* Murr., Lloydia **5**: 145. 1942. (!)
?*Volvaria fimetaria* (Murr.) Murr., Lloydia **5**: 157. 1942.
Volvariopsis griseiceps Murr., Lloydia **8**: 280. [1946.] (!)
Volvaria griseiceps (Murr.) Murr., Lloydia **8**: 290. [1946].

FIG. 10

Pileus 2–6 cm broad, obtusely conical to convex, often subumbonate, at times expanding to almost plane, dry, prominently fibrillose, with the margin not striate but often lacerate, gray with avellaneous areas or grayish avellaneous overall; flesh thin, 1 mm or less thick, with taste and odor slight or none, white. Lamellae close to subdistant, moderately broad, 4–7 mm broad, ventricose, free, with minutely fimbriate edges, whitish, then salmon-colored. Stipe 3.5–6.5 cm × 3–7 mm, enlarging to subbulbous base, terete, solid, glabrous, white. Volva up to 1 cm deep, cup-shaped, with 3–5 lobes, externally brown or grayish avellaneous, fading to whitish, white internally.

Spores 5.6–8.7 (av. 6.3–7.3) × 4.1–6 (av. 4.4–5.4) μ, ovoid to oval, occasionally subglobose or obovoid, $Q = 1.05–1.75$ (av. 1.24–1.48). Basidia 23–40 × 6–10 μ, clavate, usually 4-spored, rarely 1-, 2-, or 3-spored. Pleurocystidia 23–76 × 7–30 μ, fusoid-ventricose, clavate, or ovoid, rarely fusoid, narrowly dumbbell-shaped, or subcylindrical, common. Cheilocystidia 23–79 × 8–31 μ, fusoid-ventricose, obtusely lanceoloid, clavate with the apex acute or obtuse and bearing a knob or not, occasionally fusoid, obovoid, or subcylindrical, common. Subhymenium of cells 5–26 × 4–22 μ. Lamella trama of hyphae 2–29 μ in diameter. Pileus trama of hyphae 2–46 μ in diameter. Pileus cuticle non-gelatinous, of hyphae 2–29 μ in diameter. Clamp connections absent.

HABIT AND HABITAT. Solitary to gregarious on soil.

SPECIMENS EXAMINED. [ITALY: Bresadola, 1904 (NY, CUP-A). ARGENTINA: Singer T1687, Tucumán, Dec. 30, 1951 (LIL).] NEW

YORK: Murrill, Freeville, Tompkins Co., May 30, 1898 (CUP-A 2366). 1 additional collection (NYS). FLORIDA: Murrill F19951, Gainesville, Alachua Co., Sept. 12, 1944 (FLAS, HOLOTYPE of *Volvariopsis griseiceps* Murr.). 1 additional collection (F). TENNESSEE: Hesler 12526, Knoxville, Knox Co., June 19, 1940 (MICH). Hesler, Knoxville, Knox Co., July 19, 1941 (MICH). INDIANA: O'Neal, Bloomington, Monroe Co., July, 1919 (MICH). MINNESOTA: Whetstone, Minneapolis, Hennepin Co., Sept. 26, 1906 (CUP-A 20018). 1 additional collection (CUP-A). KANSAS: Rogerson 3731, Manhattan, Riley Co., June 18, 1955 (RLS).

The type specimen is apparently not in the Berkeley Herbarium at Kew. The interpretation of this species is based mainly upon the Bresadola specimens and various references in the European literature.

Volvariella taylori differs from *V. volvacea* in its smaller stature, paler pileus color, and smaller spores and cystidia The two Hesler collections from Tennessee actually seem to resemble *V. volvacea* in macroscopic characteristics (as far as could be determined from dried material) and *V. taylori* in microscopic characteristics.

Murrill's description of *Volvariopsis fimetaria* and the study of the holotype provide only the different habitat ("on cow manure") and the slightly smaller spores [5.6–6.7 (av. 6.1) × 4–5.2 (av. 4.5)μ] to separate this species from *Volvariella taylori*.

12. VOLVARIELLA LEPIOTOSPORA Sing., Mycologia **47**: 774. 1955. (!)

Pileus 32 mm broad, convex, becoming plane, umbonate, dry, subrimulose and with radiately arranged brownish black to black fibrils except on the disc, brownish black on the disc, entirely covered by a delicate, pale stramineous, tomentulose covering. Lamellae close, free, with fimbriate edges, rose-colored. Stipe 5 cm × 2–5 mm, tapering to the apex, subpruinose, light sordid gray. Volva caldron-shaped, arachnoid, gray.

Spores 4.7–6(–7) (av. 5.5) × 3–3.7 (av. 3.3)μ, ovoid, occasionally oval, Q = 1.52–1.95 (av. 1.67). Basidia 16–20 × 6–8 μ, clavate, 4-spored. Pileus trama of hyphae 3–30 μ in diameter. Pileus cuticle non-gelatinous, of hyphae which are 4–30 μ in diameter, contain a brown sap, and have elongate to subisodiametric terminal cells, overlaid by a superficial velar layer of hyaline to subhyaline, slender hyphae. Clamp connections present but not common.

HABIT AND HABITAT. Solitary on dead roots (*Quercus virginiana*) in tropical thicket.

SPECIMEN EXAMINED. FLORIDA: Singer F1143, Matheson Hammock, Dade Co., Oct. 20, 1942 (F, HOLOTYPE).

The description of macroscopic characteristics given above is adapted from the Latin of the original description. The microscopic structures of the type specimen did not revive well, and the cystidia could not be studied. Singer described them as "cystidiis exiguis vesiculosis; cheilocystidiis clavatis, 21–26 × 7–9.6 μ."

13. VOLVARIELLA VILLOSAVOLVA (Lloyd) Sing., Lilloa **22**: 401. [1951.] (ut *villosovolva*)

Volvaria villosavolva Lloyd, Myc. Notes **1**: 31. 1899.

Volvariopsis villosavolva (Lloyd) Murr., N. Amer. Flora **10**: 142. 1917. (ut *villosovolva*)

Pileus 2.5–3.5 cm broad, campanulate, expanding to almost plane, subumbonate, dry, innately fibrillose, more or less rimose, with the margin not striate or with short striae, fuscous on the disc, elsewhere drab-gray; flesh thin, with slight odor, white. Lamellae close, moderately broad, ventricose, free, with entire edges, pinkish cinnamon. Stipe 5–6 cm × 2–4 mm, enlarging to clavate base, terete, solid, subpruinose at the apex, otherwise glabrous, white. Volva 7–10 mm high, thick, with 2–3 lobes, covered with long mycelioid hairs, white.

Spores 5.7–7.3 (av. 6.1–6.4) × 3.6–4.6 (av. 4–4.4) μ, ovoid to oval, occasionally obovoid, Q = 1.31–1.79 (av. 1.40–1.61). Basidia 20–28 × 7–10 μ, clavate, 4-spored. Pleurocystidia 34–51 × 8–22 μ, fusoid-ventricose, occasionally lanceoloid to clavate, common. Cheilocystidia 25–51 × 9–23 μ, fusoid-ventricose, occasionally clavate, abundant. Subhymenium of cells 8–25 × 5–13 μ. Lamella trama of hyphae 2–15 μ in diameter. Pileus trama of hyphae 5–25 μ in diameter. Pileus cuticle non-gelatinous, of hyphae 4–38 μ in diameter. Clamp connections absent.

HABIT AND HABITAT. Solitary, attached to dead leaves in deciduous woods.

SPECIMENS EXAMINED. VIRGINIA: Murrill, Blacksburg, Montgomery Co., July 27–Aug. 3, 1904 (NY). MICHIGAN: Wehmeyer, Ann Arbor, Washtenaw Co., July 9, 1921 (MICH).

ILLUSTRATION: Lloyd (**1899b**), fig. 2 (photograph of carpophore).

The type of this species, which was described from Ohio, is missing from the Lloyd Herbarium at BPI; no other authentic specimens could be located. The description of macroscopic characteristics given above is drawn from Kauffman's notes, which accompany the Michigan specimen and which agree very well with Lloyd's original description. However, Lloyd described the spores as "globose, 5 mc." so some doubt must remain as to the true identity of his species.

14. **Volvariella alachuana** (Murr.) comb. nov.
Volvariopsis alachuana Murr., Lloydia **5**: 145. 1942. (!)
Volvaria alachuana (Murr.) Murr., Lloydia **5**: 157. 1942.

FIG. 11

"Pileus convex to subexpanded, slightly umbonate at times, scattered, 3–5 cm. broad, surface dry, shining, finely innate-fibrillose, white, blackish on the broad disk, margin entire, fertile; context very thin, white, unchanging; lamellae free, rounded behind, broad, close, inserted, finely eroded, white to pink; . . . stipe tapering upward, smooth, glabrous, white, 4–5 × 0.3–0.8 cm.; volva trifid, umbrinous, finely tomentose, about 1.5 cm. high and 1 cm. broad." (Murrill, **1942**).

Spores 7–8.6 (av. 7.4–7.6) × 4.4–5.6 (av. 4.9–5.1) μ, usually oval to ovoid, occasionally obovoid, Q = 1.30–1.74 (av. 1.45–1.56). Basidia 21–40 × 8–11 μ, clavate, 4-spored. Pleurocystidia 35–78 × 9–31 μ, fusoid-ventricose and then at times with the neck elongate or bulbous, lanceoloid, dumbbell-shaped, clavate, or ovoid, rare or locally abundant. Cheilocystidia 30–60 × 10–28 μ, fusoid-ventricose, fusoid, dumbbell-shaped, or clavate, common. Subhymenium of cells 10–21 × 5–15 μ. Lamella trama of hyphae 4–19 μ in diameter. Pileus trama of hyphae 4–33 μ in diameter. Pileus cuticle non-gelatinous, of hyphae 5–28 μ in diameter. Clamp connections absent.

HABIT AND HABITAT. Scattered on soil in woods.

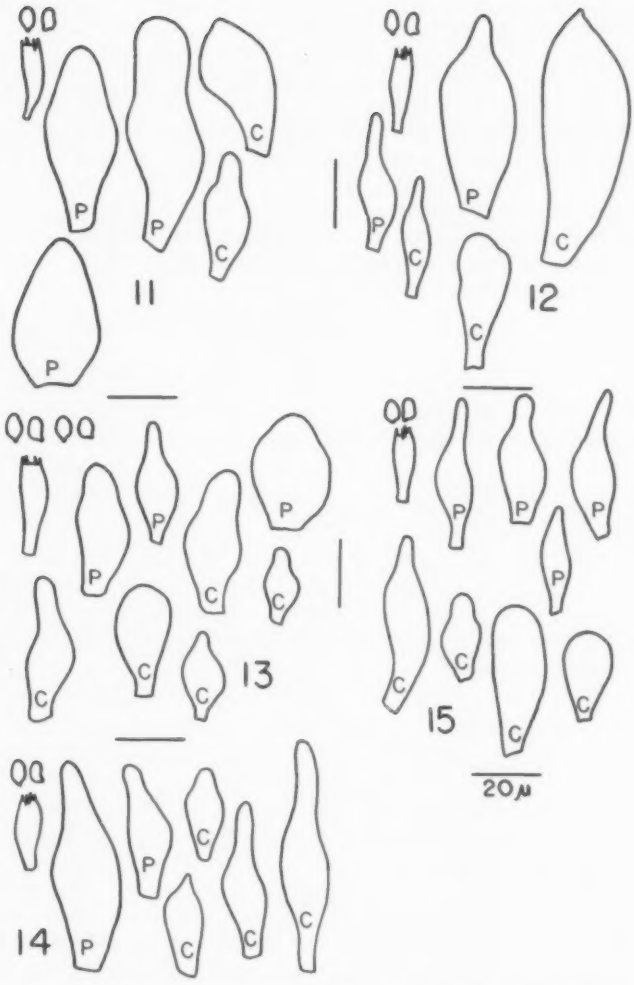
SPECIMENS EXAMINED. FLORIDA: Murrill F19655, Gainesville, Alachua Co., July 6, 1939 (FLAS, HOLOTYPE; ISOTYPES at NY and BPI). Murrill F19950, Gainesville, Alachua Co., Aug. 9, 1939 (FLAS).

15. **Volvariella smithii** sp. nov.

FIG. 12

Pileus 3.2 cm latus, obtuse conicus, siccus, glaber in centro, ceterum fibrillosus et rimosus, albidus, in centro coloris magis spadicei quam subrubicundi. Lamellae confertae, moderate latae, liberae, primoque albidae, deinde roseae. Stipes 4 cm longus, ad apicem 5 mm crassus, deorsum aliquanto crassior, dense pubescens, albidus. Volva ochracea vel cinnamomea sed pallidior. Sporae 4.7–7 × 3.1–3.9 μ. Basidia 20–27 × 6–8 μ, tetraspora. Pleurocystidia 33–79 × 6–18 μ, fusoideo-ventricosa vel clavata, numerosa. Cheilocystidia 17–67 × 8–29 μ, fusoideo-ventricosa, fusoidea, clavata, vel subcylindracea, numerosa. Hyphae non fibulatoriae.

Pileus 3.2 cm broad, obtusely conic, dry, glabrous and unpolished over the disc, appressed fibrillose and rimose along the margin, with the margin not striate, dull white except for the pinkish buff disc; flesh firm, white, with mild taste and slightly aromatic odor. Lamellae close, moderately broad, free, with slightly uneven edges, white, then pink.



FIGS. 11–15. *Volvariella.* Spores, basidia, pleurocystidia (P), and cheilocystidia (C). 11. *V. alachuana.* 12. *V. smithii.* 13. *V. pusilla.* 14. *V. hypopithys.* 15. *V. surrecta.*

Stipe 4 cm long, 5 mm thick at the apex, slightly enlarged downward, densely pubescent, dull white. Volva 5-lobed, membranous, matted fibrillose externally, ochraceous to light cinnamon color.

Spores 4.7–7 (av. 5.6) × 3.1–3.9 (av. 3.3–3.4) μ, ovoid to oval, occasionally obovoid, $Q = 1.38–1.96$ (av. 1.64–1.69). Basidia 20–27 × 6–8 μ, clavate, 4-spored. Pleurocystidia 33–79 × 6–18 μ, usually fusoid-ventricose and then at times with the neck short or thick and acute at the apex or elongate, occasionally lanceoloid to clavate, abundant. Cheilocystidia 17–67 × 8–29 μ, fusoid-ventricose, fusoid, clavate, or subcylindrical, abundant. Subhymenium of cells 4–23 × 4–15 μ. Lamella trama of hyphae 3–20 μ in diameter. Pileus trama of hyphae 4–29 μ in diameter. Pileus cuticle non-gelatinous, of hyphae 5–16 μ in diameter. Clamp connections absent.

HABIT AND HABITAT. Solitary to scattered on soil and humus in mixed woods.

SPECIMENS EXAMINED. WASHINGTON: Smith 17944, Olympic Mts., Oct. 15, 1941 (MICH). Smith 31183, south boundary of Mt. Rainier Natl. Pk., Sept. 13, 1948 (MICH, HOLOTYPE).

16. VOLVARIELLA PUSILLA (Pers. ex Fr.) Sing., Lilloa **22**: 401. [1951.]
[*Amanita pusilla* Pers., Obs. Myc. **2**: 36. 1799.] (!)
Agaricus pusillus (Pers.) ex Fr., Syst. Myc. **1**: 279. 1821.
Agaricus parvulus Weinm., Hymen. Gaster. Rossico 238. 1836.
Volvaria parvula (Weinm.) Kummer, Führ. Pilzk. 99. 1871.
Volvaria pusilla (Pers. ex Fr.) Schroet., Krypt.-flora Schles. **3**(1): 621. 1889.
Volvaria striatula Pk., Bull. Torrey Bot. Cl. **22**: 487. 1895. (!)
Volvaria umbonata Pk., Bull. Torrey Bot. Cl. **26**: 64. 1899. (!)
Volvariopsis pusilla (Pers. ex Fr.) Murr., N. Amer. Flora **10**: 141. 1917.
Volvariopsis umbonata (Pk.) Murr., N. Amer. Flora **10**: 141. 1917.
Volvariella parvula (Weinm.) Speg., Bol. Acad. Cienc. Córdoba **28**: 309. 1926.

FIG. 13

Pileus 0.5–3 cm broad, ovoid when young, expanding to campanulate-convex or convex and eventually nearly plane, umbonate or not, slightly viscid at first, soon dry, appressed-fibrillose, with the margin becoming striate, white, occasionally tinged with gray especially on the disc; flesh thin, up to 1.5 mm thick, with slight or no odor and taste, white or tinged with pink. Lamellae close to subdistant, moderately broad, up to 4 mm broad, free, with entire edges, white, becoming pink. Stipe 1–5

cm × 1–5 mm, equal or tapering to apex, terete, solid, becoming hollow, innately fibrillose, glabrous, white, at times tinged with gray. Volva 2- to 5-lobed, white, becoming grayish.

Spores (4.6–)5.4–7.9(–8.7) (av. 6.3–7.8) × (3.4–)4–5.7(–6.4) (av. 4–5.4) μ, usually ovoid to oval, occasionally subglobose or obovoid, Q = 1.10–2.07 (av. 1.24–1.69). Basidia 22–34(–37) × 7–11 μ, clavate, 4-, rarely 2-spored. Pleurocystidia 24–76 × 7–24 μ, usually fusoid-ventricose (and then at times with the neck elongate, enlarged or branched at the apex, or constricted at the base) or clavate (and then occasionally with a small knob or finger-like projection at the apex), at times dumbbell-shaped, lanceoloid, or ovoid, common to abundant. Cheilocystidia 21–61 × 7–23 μ, similar to pleurocystidia in shape, common. Subhymenium of cells 5–28 × 2–15 μ. Lamella and pileus trama of hyphae 2–28 μ in diameter. Pileus cuticle slightly gelatinous or non-gelatinous, of hyphae 2–38 μ in diameter which end in cells variously enlarged and constricted along their length. Clamp connections absent.

HABIT AND HABITAT. Solitary to gregarious on soil in lawns, gardens, and greenhouses; occasionally along paths or on bare soil in wooded areas.

SPECIMENS EXAMINED. [Persoon H.L.B. 910.255–169 (L, HOLOTYPE).] ONTARIO: Güssow, Ottawa, Aug. 19, 1932 (DAOM F5721). 1 additional collection (NY). MASSACHUSETTS: Davis, Stowe, Middlesex Co., July 17, 1907 (NYS). 1 additional collection (MICH). NEW YORK: Walker, Ithaca, Tompkins Co., July 18, 1917 (CUP-A 24205). DISTRICT OF COLUMBIA: Shear 911, Washington, Oct. 6, 1900 (BPI). 7 additional collections (BPI, MICH, NY). CAROLINA: Ravenel (Fungi Caroliniani, fasc. 1, no. 3), 1852 (BPI, NY). OHIO: Lloyd 3665 (NYS, HOLOTYPE of *Volvaria umbonata* Pk.). 1 additional collection (BPI). MICHIGAN: Fischer, Detroit, Wayne Co., 1910 (MICH, NYS). 1 additional collection (MICH). INDIANA: Jackson, West Lafayette, Tippecanoe Co., June 20, 1916 (CUP-A). MISSOURI: Glatfelter 1073, St. Louis, St. Louis Co., Aug. 10, 1903 (NYS). KANSAS: Rogerson 3674, Manhattan, Riley Co., June 14, 1954 (RLS). Bartholomew 1854, Rooks Co., July 26, 1895 (NYS, HOLOTYPE of *Volvaria striatula* Pk.; ISOTYPE at NY). 5 additional collections (BPI, NY, RLS).

ILLUSTRATIONS. Coker (**1947**), pl. 29 (photograph of carpophores); pl. 32, figs. 10–11 (drawings of spores and basidia). Hard (**1908**), fig. 194 (photograph of carpophores of *Volvaria umbonata* Pk.) and 195 (photograph of carpophores). Kauffman (**1918**), pl. 101 (photograph of carpophores). Lloyd (**1899b**), fig. 1 (photograph of carpophores).

Peck's *striatula* was based on a collection with prominently striate

pilei and broad (5.3–6.4 μ) spores, and his *umbonata,* on a collection with strongly umbonate and striate pilei. None of these characteristics are sufficiently constant and distinctive to permit the recognition of Peck's species.

See also the discussion following *Volvariella hypopithys.*

17. **Volvariella hypopithys** (Fr.) comb. nov.

Agaricus hypopithys Fr., Hymen. Europ. 183. 1874.

Volvaria plumulosa (Lasch) ex Quél., Bull. Soc. Bot. Fr. **24**: 320. 1877.

Agaricus (*Volvaria*) *pubescentipes* Pk., 29th Rep. N. Y. St. Mus. (1875) 39. 1878. (!)

Volvaria hypopithys (Fr.) Karst., Bidr. Finl. Nat. Folk **37**: 251. 1879.

Volvaria pubipes Sacc., Syll. Fung. **5**: 658. 1887. (ut *V. pubipes* Pk.)

Volvaria pubescentipes (Pk.) Lloyd, Comp. Volvae 11. 1898.

Volvaria perplexa Pk., N. Y. St. Mus. Bull. 167, p. 49. 1913. (!)

Volvariopsis perplexa (Pk.) Murr., N. Amer. Flora **10**: 141. 1917.

Volvariopsis pubescentipes (Pk.) Murr., N. Amer. Flora **10**: 141. 1917.

Volvariella plumulosa (Lasch ex Quél.) Sing., Lilloa **22**: 401. [1951.]

Volvariella pubescentipes (Pk.) Sing., Lilloa **22**: 401. [1951.]

FIG. 14

Pileus 2–5 cm broad, campanulate or convex and expanding to nearly plane, at times subumbonate, dry, innately fibrillose and silky, becoming squamulose, with the margin fimbriate and not striate or only slightly so, white, the disc at times yellowish; flesh thin, with taste and odor slight or none, white. Lamellae close to crowded, narrow, free, with the edges whitish-fimbriate, white, then flesh color. Stipe 2–8 cm × 2–4 mm, subequal to equal, terete, with the base more or less bulbous, solid, becoming hollow, densely pubescent to villose, glabrescent below, white. Volva membranous, 2- to 4-lobed, white.

Spores (5.4–)6–8.6(–9.9) (av. 6–8) × (3.3–)4.1–6 (av. 4.2–5.2) μ, ovoid to oval, occasionally obovoid, rarely subglobose, $Q = 1.13–1.87$ (av. 1.38–1.78). Basidia 21–34 × 7–10 μ, clavate, 4-, rarely 2-spored. Pleurocystidia 14–70 × 7–33 μ, fusoid to fusoid-ventricose and then at times with the neck elongate or enlarged at the apex or constricted at the base, lanceoloid, clavate with the apex acute or obtuse, more or less dumbbell-shaped, ovoid, or rarely subcylindrical, usually common, but

rare in some sections. Cheilocystidia 22–90(–124) × 7–36 μ, usually fusoid-ventricose or clavate (and then with the apex acute or obtuse and bearing a knob or not), occasionally ovoid or obovoid, fusoid, lanceoloid, subcylindrical, or dumbbell-shaped, usually common. Subhymenium of cells 5–25 × 2–15 μ. Lamella trama of hyphae 2–32 μ in diameter. Pileus trama of hyphae 2–35 μ in diameter. Pileus cuticle non-gelatinous, of hyphae 2–35 μ in diameter which terminate in cells variously enlarged or constricted along their length. Clamp connections absent.

HABIT AND HABITAT. Solitary to scattered on humus in coniferous, deciduous, and mixed woods.

SPECIMENS EXAMINED. [ITALY: Bresadola, Trento (NY). Bresadola, 1890 (CUP-A).] ONTARIO: Groves, Ottawa, Sept. 8, 1945 (DAOM 17136). 2 additional collections (DAOM). MASSACHUSETTS: Hill, Boston, Sept. 18, 1910 (MICH). NEW YORK: Atkinson, Lake Placid, Essex Co., Aug. 27–Sept. 3, 1898 (CUP-A). Peck, Sandlake, Rensselaer Co., Aug. (NYS, HOLOTYPE of *Agaricus pubescentipes* Pk.). Atkinson 5642, Ithaca, Tompkins Co., Oct., 1900 (CUP-A). 13 additional collections (CUP, CUP-A, NYS). PENNSYLVANIA: Delafield 34, Buck Hill Falls, Monroe Co., Oct. 1, 1920 (NY). MICHIGAN: Stuntz (Smith 33339), Tahquamenon Falls, Luce Co., Sept. 3, 1949 (MICH). Smith 25743, Douglas Lake, Cheboygan Co., July 15, 1947 (MICH). Kauffman, Bay View, Emmet Co., July 18, 1905 (MICH). Smith 1793, George Reserve, Livingston Co., Aug. 10, 1935 (MICH). 22 additional collections (DAOM, MICH). MINNESOTA: Whetstone, 1911 (NYS, HOLOTYPE of *Volvaria perplexa* Pk.). CALIFORNIA: Smith 9067, Crescent City, Del Norte Co., Nov. 24, 1937 (MICH). Morse 265, Berkeley, Alameda Co., Jan. 5, 1928 (BPI).

ILLUSTRATIONS. Coker (**1947**), pl. 32, figs. 12–15 (drawings of carpophore, spores, basidium, and cystidia). Peck (**1878**), pl. 1, figs. 1–3 (drawings of carpophores and spores of *Agaricus pubescentipes* Pk.).

The identity of the species having the epithets *hypopithys, plumulosa,* and *pubescentipes* has evidently plagued practically every mycologist who has worked with *Volvariella.* After examination of numerous specimens from both North America and Europe, it seems most logical at this time to recognize these taxa as constituting one variable species to which the oldest available epithet is applied. A difference in habitat has been the most constant distinction used by European authors to distinguish *hypopithys* (coniferous woods) from *plumulosa* (deciduous woods). No morphological differences could be correlated with this in the European material and in the American material identified by

European mycologists (principally Bresadola). Peck (**1878**) evidently considered the squamulose pileus with fimbriate margin to separate *pubescentipes* from *hypopithys*, but this does not hold. The reportedly glabrous stipe and absence of cystidia would provide differences for *Volvaria perplexa* (Peck, **1913**), but in the dried holotype at least one stipe is definitely pubescent on the upper portion and cystidia are present, though rare. *Volvariella pusilla* and *V. hypopithys* may also not be so distinct as indicated in the key; however the relationships here must await the collection of more specimens.

18. VOLVARIELLA SURRECTA (Knapp) Sing., Lilloa **22**: 401. [1951.]
Agaricus surrectus Knapp, "J. Naturalist, p. 363. 1829." (fide Ramsbottom, **1942**)
Agaricus loveianus Berk. in J. E. Smith, Engl. Flora **5**(2): 104. 1836. (!)
Volvaria loveiana (Berk. in J. E. Smith) Gill., Les Champ. 386. 1878.
Volvaria hypopithys (Fr.) Karst. subsp. *loveiana* (Berk. in J. E. Smith) Konrad & Maubl., Icon. Sel. Fung. **1**: pl. 17, fig. 2. 1925. (ut *loweana*)
Volvaria surrecta (Knapp) Ramsb., Trans. Brit. Mycol. Soc. **25**: 326. 1942.

FIG. 15

Pileus 2.5–8 cm broad, ovoid when young, becoming campanulate or convex and finally nearly plane, broadly umbonate or not, dry, silky fibrillose, with the margin not striate, white to light gray, with the disc becoming yellowish or brownish or not; flesh moderately thick, soft, white, with slight odor and mild taste. Lamellae close, moderately broad, subventricose, free, white-floccose on the edges, white, becoming deep flesh pink. Stipe 4–9 cm × 4–12 mm, equal or enlarging to base, terete, solid, fleshy-fibrous, appressed fibrillose, pruinose at the apex, white to light gray. Volva 1.3–2.5 cm high, 0.6–1.3 cm broad, with the free margin lobed or nearly even, white.

Spores 5.4–7.6 (av. 6.4–6.9) × 3.4–4.9 (av. 3.9–4.3) μ, ovoid to oval, occasionally obovoid, Q = 1.36–1.96 (av. 1.54–1.67). Basidia (17–)20–31 × 5–10 μ, clavate, 4-spored. Pleurocystidia 21–57 × 8–29(–38) μ, usually fusoid-ventricose and then at times with the neck elongate or enlarged at the apex, occasionally fusoid, clavate, or ovoid, common. Cheilocystidia 25–50(–70) × 6–20(–39) μ, fusoid-ventricose with the neck at times short and bulbous, fusoid, lanceoloid, clavate, or obovoid, abundant. Subhymenium of cells 5–18 × 4–13 μ. Lamella trama of hyphae 2–18 μ in diameter. Pileus trama of hyphae 2–36 μ in diameter.

Pileus cuticle non-gelatinous, of hyphae 5–29 μ in diameter. Clamp connections absent.

HABIT AND HABITAT. Solitary to gregarious and parasitic on *Clitocybe* spp. (usually *C. nebularis*).

SPECIMENS EXAMINED. [ENGLAND: Berkeley, Wothorpe, Northants., 1833 (K, HOLOTYPE of *Agaricus loveianus* Berk.; here designated NEOTYPE of *Volvariella surrecta*).] ONTARIO: Dearness 2426, London, Oct. 3, 1896 (NY, CUP-A, BPI). Dearness (Ellis & Everhart, N. Amer. Fungi, ser. 2, 3509), London, Oct., 1896 (BPI, CUP-A, F). MINNESOTA: Whetstone, Minneapolis, Hennepin Co., Oct. 15, 1915 (F1124768).

ILLUSTRATIONS. Harper (**1916**), pl. 177, fig. A, B (photographs of carpophores). Smith ([**1934**]), pl. 44 (photograph of carpophores).

IMPERFECTLY KNOWN SPECIES

19. VOLVARIA AVELLANEA (Clem.) Kauff., Pap. Mich. Acad. Sci. **4**: 335. 1924.

Lepiota avellanea Clem., Bot. Surv. Neb. **2**: 41. 1893.

"Pileus fleshy, dry, plane, drab-colored, cuticle lacerate toward the margin, forming appressed brown scales; stipe somewhat hollow, bulbous, brownish-fibrillose; annulus small, fleshy, concolorous, fixed, inferior; lamellae remote, attached to an indistinct collar, cream-colored, becoming reddish with age; spores irregularly ovate, acute at one end, 8–10 × 5–6 μ.

"Pileus 5 cm. wide, stipe 4 cm. long by 8 mm. wide. On ground in greenhouse, Lincoln. (547)" (Clements, **1893b**).

The type specimens of the four species described by Clements (*Lepiota avellanea, Volvaria concinna, V. submyochroa,* and *V. viscosa*) are neither at NEB nor at BPI, and Mr. John Stevenson (in litt.) believes them to be no longer in existence. Kauffman (**1924**) published an emended description of *Volvaria avellanea,* which he reportedly observed and studied frequently in Washington, D. C., but his specimens also have not come to light. He cited *Volvaria concinna* as a synonym of *V. avellanea.*

According to Kauffman's description, the vinaceous-brown, scaly pileus, the vinaceous-brown volva which frequently disappears early leaving its upper portion as a narrow "annulus," and the relatively large spores (9–12 × 5–7 μ) should make *Volvaria avellanea* easily recognizable.

20. VOLVARIA CINEREA Beardslee, J. Elisha Mitchell Sci. Soc. **31**: 148. 1915.

"Pileus 1–2 cm. broad, gray or bluish gray, soon plane, with a small rounded umbo, clothed with long, dark, appressed fibrils, striate on the margin.

"Gills rounded behind, remote from the stipe, white, then flesh color.

"Stipe white, solid, fibrillose.

"Volva dark-colored, splitting into three or four divisions.

"Spores 5–6 mc. long, broadly elliptic.

"Growing on rotting logs of deciduous woods." (Beardslee, **1915**).

This species was described from a collection made at Asheville, N. C. Neither type nor authentic specimens could be located.

21. VOLVARIA CONCINNA Clem., Bot. Surv. Neb. **5**: 9. 1901.
Volvariopsis concinna (Clem.) Murr., N. Amer. Flora **10**: 142. 1917.

"Pileus submembranaceous, expanded, not at all or only slightly umbonate, smooth, pale avellaneous, ½–1½ cm. wide; stipe graceful, concolorous, 1–2 mm. × ½–1½ cm.; volva minute, strictly adpressed, limb obsolescent; lamellae free, rose-colored; spores ovate-ellipsoid, granular or guttate, smooth, rosy, 8–11 × 5–7 μ; cystidia lacking.

"On moist shaded ground and on flooded banks, Nemaha River, Humboldt, 1897; Marysville, Kansas, 1896. (12110)" (Clements, **1901**).

See the discussion following *Volvaria avellanea*.

22. VOLVARIA SUBMYOCHROA Clem., Bot. Surv. Neb. **5**: 10. 1901. (ut *submyochrous*)
Volvariopsis submyochroa (Clem.) Murr., N. Amer. Flora **10**: 142. 1917.

"Pileus subcarnose, convex, almost plane, scarcely umbonate, silky, shining, umbo densely covered with larger silky fibrils, becoming innate towards the strongly striate margin, avellaneo-murinous, 3–4 cm. wide; stipe carnose, equal, solid, white, shining, farinose at apex, 5 mm.× 3–4 cm.; volva small, hirsute, 2–3-fid, concolorous; lamellae free, remote, subconfertous, ventricose, at first flesh-colored, then isabel-colored; spores ellipsoid, uniguttate, smooth, 6–7 × 4 μ.

"On wet earth in basement, University Campus, Lincoln. (12111)" (Clements, **1901**).

See the discussion following *Volvaria avellanea*.

23. VOLVARIA VISCOSA Clem., Bot. Surv. Neb. **2**: 37. 1893.
Volvariopsis viscosa (Clem.) Murr., N. Amer. Flora **10**: 143. 1917.

"Pileus fleshy, campanulate-convex, smooth, very viscous, fulvous-ochraceous; stipe prominently bulbous, nearly equal above, solid, smooth, ochraceous; volva ample, lobed, concolorous; lamellae touching, brown; spores ovoid-ellipsoid, dilutely flesh-colored, with a large locule, $8 \times 5\ \mu$. Pileus 6 cm. wide; stipe 6 cm. long, at base $1\frac{1}{2}$ cm. wide, above $\frac{1}{2}$ cm. Related to *V. primulina* Cooke & Massee.

"Warbonnet canon. (505)" (Clements, **1893a**).

See the discussion following *Volvaria avellanea*. *V. viscosa* would seem distinct among the North American species on the basis of its viscid, fulvous-ochraceous pileus, ochraceous stipe and volva, and small spores.

ACKNOWLEDGMENTS

Persons connected with several herbaria were very cooperative in loaning specimens or supplying information about them. Their names are here listed in appreciation of the favors they granted: Dr. Charles Baehni, Conservatoire et Jardin Botaniques, Genève (G); Dr. John F. Davidson, University of Nebraska State Museum (NEB); Dr. R. W. G. Dennis, Royal Botanic Garden, Kew (K); Dr. Francis Drouet, Chicago Natural History Museum (F); Dr. R. A. Maas Geesteranus, Rijksherbarium, Leiden (L); Dr. J. Walton Groves, Mycological Herbarium, Ottawa (DAOM); Miss E. Kathleen Goldie-Smith, University of North Carolina (NCU); Dr. Richard P. Korf, Cornell University (CUP); Dr. I. Mackenzie Lamb, Farlow Herbarium, Cambridge (FH); Dr. Juan C. Lindquist, Instituto de Botánica C. Spregazzini, La Plata (LPS); Dr. W. Robyns, Jardin Botanique de l'Etat, Bruxelles (BR); Dr. Donald P. Rogers, New York Botanical Garden (NY); Dr. Clark T. Rogerson, Kansas State College (KSC); Dr. Rolf Singer, Instituto Miguel Lillo, Tucumán (LIL); Dr. A. H. Smith, University of Michigan (MICH); Mr. Stanley J. Smith, New York State Museum, Albany (NYS); Mr. John A. Stevenson, National Fungus Collections, Beltsville (BPI); and Dr. Erdman West, University of Florida (FLAS). Dr. Richard T. Bruère of the University of Chicago kindly provided help with the Latin description.

DEPARTMENT OF PLANT PATHOLOGY
CORNELL UNIVERSITY
ITHACA, NEW YORK

LITERATURE CITED

Atkinson, G. F. 1900. Mushrooms, Edible, Poisonous, etc. Ithaca, Andrus and Church. vi + 275 p., 223 fig.

Battarra, J. A. 1755. Fungorum agri ariminensis historia. Faventiae, Typis Ballantianus Superiorum Permissu. vii + 80 p., 40 pl.

Beardslee, H. C. 1915. Notes on new and rare species of fungi found at Asheville, N. C. Jour. Elisha Mitchell Sci. Soc. **31**: 145–149.

Briquet, J. 1935. International Rules of Botanical Nomenclature. Ed. 3. Jena, Gustav Fischer. xi + 152 p.

Clements, F. E. et al. 1893a. Notes on the canon flora of Sioux County, with list of plants collected in July and August, 1892, by A. F. Woods. Bot. Surv. Neb. **2**: 31–38.

——. 1893b. Miscellaneous additions to the flora of the state, and new or noteworthy species from various localities. Bot. Surv. Neb. **2**: 39–46.

——. 1901. Bot. Surv. Neb. **5**: 1–143.

Coker, W. C. 1947. North Carolina species of *Volvaria*. Jour. Elisha Mitchell Sci. Soc. **63**: 220–230, pl. 28–32.

Donk, M. A. 1941. Nomina generica conservanda and confusa for Basidiomycetes (fungi). Bull. Bot. Gard. Buitenz. III. **17**: 155–197.

——. 1949. New and revised nomina generica conservanda proposed for Basidiomycetes (fungi). Bull. Bot. Gard. Buitenz. III. **18**: 83–168.

Earle, F. S. 1909. The genera of North American gill fungi. Bull. N. Y. Bot. Gard. **5**: 373–451.

Fries, E. 1821. Systema mycologicum. Vol. 1. Gryphiswaldiae, Ernesti Mauritii. lvii + 520 p.

Hard, M. E. 1908. The Mushroom. Columbus, The Ohio Library. xii + 609 p., 504 fig.

Harper, E. T. 1916. Two parasitic mushrooms. Mycologia **8**: 65–72, pl. 177–179.

Kauffman, C. H. 1918. The Agaricaceae of Michigan. Lansing, Mich. Geol. Biol. Surv. Publ. 26, Biol. Ser. 5. Vol. 1. Text. xxvii + 924 p. Vol. 2. Plates. 172 pl.

——. 1924. The genus *Lepiota* in the United States. Pap. Mich. Acad. Sci. **4**: 311–344, pl. 15–18.

Kummer, P. 1871. Der Führer in die Pilzkunde. Zerbst, E. Luppe. 146 p.

——. 1882. Der Führer in die Pilzkunde. Ed. 2. Zerbst, E. Luppe. 188 p., 4 pl.

Kuntze, O. 1891. Revisio generum plantarum. Part 2. Leipzig, Arthur Felix. P. 377–1011.

Lamarck, J. B. A. P. M. de and A. P. De Candolle. 1805. Flore française. Ed. 3. Vol. 2. Paris, Desray. xii + 600 p.

Lanjouw, J. and F. A. Stafleu. 1956. Index herbariorum. Part I. The herbaria of the world. Ed. 3. Utrecht. 224 p.

Lloyd, C. G. 1898. A compilation of the volvae of the United States. Mycol. Writings **1**. 22 p.

——. 1899a. The small Volvarias. Mycol. Writings **1**. Mycol. Note 2: 9–10.

——. 1899b. The genus *Volvaria* again. Mycol. Writings **1**. Mycol. Note **4**: 31, 2 fig.

McIlvaine, C. 1900. One Thousand American Fungi. Indianapolis, Bowen-Merrill Co. xxxvii + 704 p., 181 pl.

Murrill, W. A. 1911. The Agaricaceae of tropical North America. Mycologia **3**: 271–282.

——. 1917. *Volvariopsis* Murrill. N. Amer. Flora **10**: 140–145.

——. 1938. New Florida agarics. Mycologia **30**: 359–371.

——. 1942. New fungi from Florida. Lloydia **5**: 136–157.

——. 1949. Three new fleshy fungi. Mycologia **41**: 490–491.

——. 1951. Florida rosy-spored agarics. Lloydia **14**: 194–200.

Patouillard, N. 1900. Essai taxonomique sur les familles et les genres des hyménomycètes. Lons-le-Saunier, Lucien Declume. 184 p.

Peck, C. H. 1878. 29th Rep. N. Y. St. Mus. (1875), p. 29–82, 2 pl.

——. 1896. Annual report of the botanist. 48th Rep. N. Y. St. Mus. (1894), 241 p., A + 43 pl.

——. 1913. Report of the state botanist, 1912. N. Y. St. Mus. Bull. 167. 137 p., pl. 131–132, 9–10.

Quélet, L. 1872. Les champignons du Jura et des Vosges. Mém. Soc. Emul. Montbéliard II. **5**: 42–332, 23 + 1 pl.

Ramsbottom, J. 1942. *Volvaria surrecta* (Knapp) comb. nov. Trans. Brit. Mycol. Soc. **25**: 326–328, 1 fig.

Rogers, D. P. 1949. Nomina conservanda proposita and nomina confusa—fungi. Farlowia **3**: 425–493.

——. 1950. Nomina conservanda proposita and nomina confusa—fungi. Supplement. Farlowia **4**: 15–43.

——. 1953. Disposition of nomina generica conservanda for fungi. Taxon **2**: 29–32.

Singer, R. 1950. Type studies on Basidiomycetes, IV. Lilloa **23**: 147–246.

——. [1951a.] The "Agaricales" (mushrooms) in modern taxonomy. Lilloa **22**: 1–832, 29 pl.

——. 1951b. Die Nomenklatur der höheren Pilze. Schweiz. Z. Pilzk. **29**: 204–228.

——. 1955. Staude redivivus. Mycologia **47**: 270–272.

—— **and A. H. Smith.** 1946. Proposals concerning the nomenclature of the gill fungi including a list of proposed lectotypes and genera conservanda. Mycologia **38**: 240–299.

Smith, A. H. [1934.] Unusual agarics from Michigan. Pap. Mich. Acad. Sci. **19**: 205–215, pl. 36–44.

——. 1945. Volvaria bombycina. Mycologia **37**: 440–443, 1 fig.

——. 1949. Mushrooms in Their Natural Habitats. Vol. 1. Portland, Sawyer's Inc. xiv + 626 p., 11 fig.

Spegazzini, C. [1899.] Fungi argentini novi v. critici. An. Mus. nac. B. Aires **6**: 81–365, pl. 4–5.

Stevenson, J. A. 1953. A list of authors of plant parasite names with recommended abbreviations. Plant Dis. Surv. Spec. Publ. 1 (index part): 1233–1263.

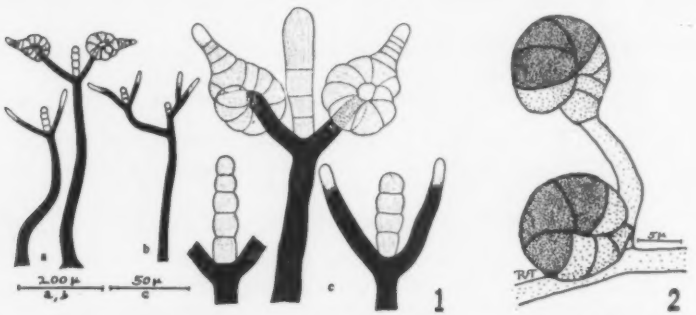
Wakefield, Elsie M. 1939. Nomina generica conservanda. Contributions from the nomenclature committee of the British Mycological Society I. Trans. Brit. Mycol. Soc. **23**: 215–232.

INDEX TO THE HELICOSPORAE: ADDENDA

ROYALL T. MOORE

(WITH 4 FIGURES)

Since publishing the Index (**6**), eleven new Helicosporae and several new distribution records have come to my attention. Glen-Bott (**3**) adds three new species to the genus *Helicodendron*. She includes in her paper a key to the hyaline and subhyaline species, in which the primary separations are based on characteristics of these fungi when grown on malt agar. She presents morphological descriptions of all but one of these, making it unnecessary, I feel, to include them here. Of the remaining eight species, four are from the literature and four are reported



FIGS. 1, 2. Helicosporae. 1. *Circinoconis paradoxa*. a. Conidiophores, the right one bearing conidia. b. Abnormal conidiophore. c. Tops of conidiophores (from Boedijn). 2. *Helicoma Isiola*, portion of a glomerula.

here for the first time. Within the former group, *Circinoconis paradoxa* Boedijn (**1**) is the type of a new genus. Boedijn's first figure (FIG. 1) is reproduced here, but it must be noted that the magnifications given on the published plates yield measurements which must be halved to comply with the diagnosis. The added scales have been prepared accordingly.

Concerning the latter group and the new distributions: helicosporous material sent me by Mr. Robert J. Bandoni of the State University of Iowa included a specimen (no. *55–7*) of *Helicoma stigmateum* (Reiss)

Linder collected at Iowa City. To the best of my knowledge this is the fourth known collection of this species, and the first in this country—the others, including the type, are from Europe. Its scarcity may well be due not to rarity but to the fact that it is so diminutive that even under the high power of the dissecting microscope it appears as a diffuse brownish powder sparsely sprinkled across the substrate. Also, this material bears no Sphaeropsidaceae.

The other material was transmitted by Dr. D. P. Rogers of the New York Botanical Garden and it contained not only three undescribed species, but also some new distribution records:

Xenosporella Berkeleyi (Curtis) Linder. On bamboo, San Diego de los Baños, Pinar del Rio Province, Cuba, Earle and Murrill *393*.

Helicosporium panacheum Moore. On *Arecastrum* (monocot) wood, Manoa Valley, Oahu, Hawaii, I. A. Abbott *1201*. I was pleased to note that this material also was characterized by coattached conidia, which gives to them the distinctive panache or plumed appearance.

Helicosporium Hendrickxii Hansford. From Njala, Sierra Leone, on *Elaeis guineensis* and *Wallichia disticha*, F. C. Deighton *1735* and *1705* respectively.

It should be noted that none of these is parasitic and at the time of collection they were growing saprobically on the respective substrates.

Finally, authentic material of the type of *Drepanospora* Berkeley & Curtis from the Curtis Herbarium, Farlow, was examined. A summary and disposition of this monotypic genus follows.

DREPANOSPORA Berkeley and Curtis

1875. Berkeley (**2**) proposed this name for his single species *Drepanispora pannosa* and he described it as being "pannosa, floccis flexuosis, his illic processibus brevibus fertilibus praeditis; sporis longissimis insigniter falcaltis pluriseptatis," and he designated Car. Inf. *2354* as the type.

1910. Von Höhnel (**4**), in discussing this species, concluded that *pannosa* ". . . ist nichts anders als ein *Helicosporium* mit nur halbkreisförmig gebogenen Sporen und es ist fraglich, ob die Gattung *Drepanospora* aufrecht zu erhalten ist." However, he did not effect the transfer.

1953. Hughes (**5**) in a footnote states that ". . . three species included in *Helicosporium* by Linder are considered best classified in the apparently dry-spored genus *Drepanospora* [*Drepanispora*] Berk. & Curt., e.g. *D. pannosa* Berk. & Curt. (= *Helicosporium serpentinum* Linder), and the *Drepanospora* conidia of *Lasiosphaeria nematospora*

Linder [*H. nematosporum* Linder] and *L. elinorae* Linder [*H. Elinorae* Linder]."

One interesting point is that the Berkeley and Curtis sheet, and the sheet with the type material of *Heliocosporium nematosporum* Linder bear notations equating these specimens to *Helicosporium pannosa* [sic] and crediting von Höhnel with the transfer. The writing is Linder's beyond a reasonable doubt, but though he made a slide (no. *41213*), Linder apparently did not disagree with either von Höhnel or Hughes. Examination of authentic and type material of *pannosa, nematosporum,* and *serpentinum* demonstrates these to be distinct species within the genus *Helicosporium.*

Helicosporium pannosum (Berk. & Curt.) comb. nov.
≡ *Drepanispora pannosa* Berk. & Curt., Grevillea **3**: 105. 1875. Car. Inf. *2354.*

Colonies forming an effuse brown layer over the substrate; conidiophores extending up to 130 μ high, 7–8 μ thick, simple or sparsely branched, surface slightly papulose-roughened, bearing the conidia on stout teeth 5–5.5(–10.5) × 3–4 μ; filaments hyaline, strongly curved to once spiraled, brittle, 5.5–7.5 μ thick. The somatic mycelium also produces long, simple, quite fuscous, septate, sterile processes up to 140–160 μ long, which taper slightly toward the blunted tips.

The following are the diagnoses of the four new species:

Helicosporium Neesii sp. nov.

Coloniis effusis, conidiis oculo nudo fumosis, conidiophoris fuscis; conidiis hyalinis pleurogenis in partibus inferioribus conidiophorum, singulatim ad vesiculos prolatis, 2–4.5 × 4.5–8 μ; conidiophoris erectis, 350–400 μ altis, apicem versus angustioribus, flexuosis, saepe in parte apicali ramosis; filamentis tenellis, 1–2 μ crassis, multiseptatis, tenus quinquies contortus, diametro spirarum 13–18 μ.

In ligno putrido, Newfield, New Jersey, 8 June 1882, J. B. Ellis *258,* TYPUS, in herb. NY; slide RTM *1:127.*

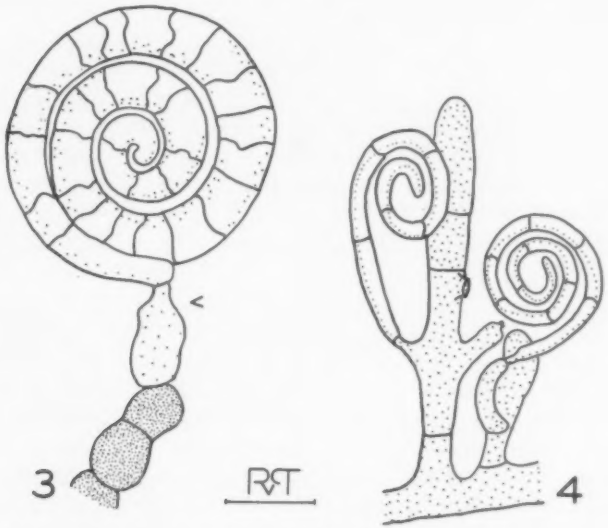
Colonies effuse, conidia in mass smoky gray, conidiophores fuscous; conidia hyaline, pleurogenous on the lower portions of the conidiophores, borne singly on bladder-like projections, 2–4.5 × 4.5–8.5 μ; conidiophores erect, 350–400 μ high, terminally tapering-flexuous, frequently branching above; filaments slender, 1–2 μ thick, multiseptate, up to 5 times coiled, diameter of spiral 13–18 μ.

(Etm. In honor of Nees von Esenbeck, who established the genus *Helicosporium.*)

This species is adjacent to *Helicosporium decumbens* Linder. How-

ever, *Neesii* is characterized by conidia that are large and confined to the lower portions of the conidiophores, and by conidiophores that are erect and tapering-flexuous above.

Also present on this material is what appears to be a species of the phaeodictyous Phomaceae.



FIGS. 3, 4. Helicosporae. 3. *Helicoma acrophalarium*, arrow indicating usual point of disjunction. 4. *Helicomyces lilliputeus*. (Scale line equals 10 μ.)

Helicomyces lilliputeus sp. nov. FIG. 4

Coloniis sparsis; conidiis hyalinis, 12.5–19 μ diametro, paucis, acropleurogenis; filamentis circiter 2 μ crassis; conidiophoris robustis, simplicibus aut ramosis, 5–6 μ crassis, usque 40 μ altis, subhyalinis.

In ligno dicotyledono putrido, Oahu, Hawaii, 11 February 1946, leg. I. A. Abbot, D. P. Rogers *653*, TYPUS, in Herb. NY; slide RTM *1:129*.

Colonies sparse; conidia hyaline, 12.5–19 μ in diameter, sparse, acropleurogenous, filaments about 2 μ thick; conidiophores stout, branched or unbranched, up to 40× 5–6 μ, subhyaline.

(Etm. Lilliputian—diminutive, in reference to the conidia.)

Lilliputeus, like *Helicomyces bellus* and *H. fuscipes* [*fuscopes*], has subhyaline conidiophores that in size are quite comparable to those of *fuscipes*. However, *lilliputeus* is quite distinct by virtue of conidia that

are considerably smaller than those of any previously described species, and, unlike *fuscipes,* it has stoutly branching conidiophores.

Helicoma acrophalerium sp. nov. FIG. 3

Colonis sparsis; filamentis basim versus angustioribus, rotundatis et oblique complanatis, multiseptatis, septis exstantibus, rugosiusculis, hyalinis, bis aut ter contortis, 5–7.5 μ crassis, diametro spirarum 26–32 μ; conidiophoris ad 10–15 μ altis, fuscis, cellula terminali nonnihil inflata excepta hyalina.

In ligno putrido, Porto Rico, 24 January ad 5 April 1923, Fred J. Seaver and Carlos E. Chardon *580,* TYPUS, in Herb. NY; slide RTM *I:128.*

Colonies sparse; filaments tapering toward the rounded, obliquely flattened basal ends, multiseptate, with salient, more or less wrinkled, septa; conidia hyaline, coiled 2–3 times, 5–7.5 μ thick, spiraled diameter 26–32 μ; conidiophore up to 10–15 μ high, fuscous, except the hyaline, somewhat inflated, terminal cell.

(Etm. Ακροφαληριάω—to be white above, in reference to the terminus of the conidiophore.)

The species *acrophalarium* is placed among the Helicomas on the basis of the thick, non-hygroscopic filaments, and the short conidiophores. While specifically distinct by virtue of the sinuous conidial septation and unique measurements, it is the prominent terminal cell of the conidiophore that is most characteristic. This is hyaline (in contrast to the fuscous subtending cells), and is joined to the conidium by a stout sterigma. There are no apparent septa separating the sterigma from either the basal cell of the conidium or the terminal cell of the conidiophore. Phloxine, however, stains only the conidial contents and to a considerably lesser degree the terminal cell, leaving the thick walls and sterigma hyaline. The sterigma, upon spore disjunction, generally remains with the conidium.

Also present on this material is a *Sporodesmium* sensu Saccardo, and what is apparently an endosporous Deuteromycete.

Helicoma Isiola sp. nov. FIG. 2

Coloniis effusis vel altilaneis; conidiis 10–17 × 7–9 μ (mediis 12.5 × 8.5 μ), cellulis proximalibus et distalibus hyalinis, mediis fuscis, per tres quartas vel semel conspiratis; conidiophoris obsoletis, conidiis directe e mycelio aut acropleurogenis a brevibus ramis paritis.

In tentorio, Oro Bay, New Guinea, 26 April 1945, QM *760,* TYPUS, in herb. Natick QM. RTM *I:130.*

Colonies effuse to densely flocculose; conidia 10–17 × 7–9 μ (avg. 12.5 × 8.5 μ), proximal and distal cells hyaline, medial cells fuscous, coiled ¾–1 times; conidiophores obsolete, conidia arising directly from the mycelium or else borne acropleurogenously on short branches.

(Etm. Dim. of *Isia,* the genus of the "wooly bear" caterpillar, in reference to the conidial pigmentation.)

This fungus grows readily on a variety of substrates; on rich media such as Steep (see Raper and Thom, *Manual of the Penicillia,* 1949, p. 67) and Potato Dextrose agars it will produce large, dense colonies four to five centimeters in diameter and as much as four millimeters in thickness. Mycelial production is definitely superordinate to the production of conidia, which tend to be produced in glomerulae. Seldom do these become very large or conspicuous. However, conidia were readily visible on a culture in which the mycelium had been greatly suppressed by growing the fungus on cornstalk in plain agar. Also, it has been found that covering portions of the colony with sterile mineral oil will increase conidial production in those areas. Furthermore, in the QM Herbarium there is a dried potato dextrose agar culture that has very prominent and distinct conidial clusters. The card on which this specimen is mounted bears the following notation: "on 0.5% pda 6 June 1947–Sept. 1953. Held at 6° C after 1948." Finally, QMD Report No. 303 for December 1945, page fifteen, reports a tensile strength loss of 38% in tested duck strips.

The following modifications to the respective keys (**6**) are needed to accommodate these additional taxa:

KEY TO THE GENERA OF THE HELICOSPORAE: ADDENDUM

10. Conidia muriform.....*Xenosporella* Höhnel
10. Conidia transversely septate.....A
A. Conidia racket-shaped, solitary, consisting of one coil of cells terminating in a rostrum; conidiophores straight, apically trifurcate, bearing the conidia only on the side furcae. With the single species *C. paradoxa* Boedijn.....*Circinoconis* Boedijn (**1**)
A. Conidia circular, borne singly or plurally, coiled once to several times; conidiophores simple to sparsely branched, not apically furcate, bearing the conidia on pegs or teeth.....*Helicoma* Corda

KEY TO THE SPECIES OF HELICOMA: ADDENDA

8′. Conidia coiled ¾–1½ times.....A
8′. Conidia coiled more than 1½ times.....9
A. Conidia hyaline throughout, 7–10 μ in diameter, 1–3-septate, ¾–1½ times coiled, 3–3.5 μ thick, may occur on Sphaeropsidaceae.....
.....8. *stigmateum* (Reiss) Linder
A. Conidia medially fuscous, the proximal and distal ends hyaline, 10–17 μ in diameter, when mature more than 3-septate, ¾–1 times coiled, 7–9 μ thick.....34. *Isiola* Moore

* * *

14′. [*H. tenuifilum* Linder]
14′. Conidiophores simple.....B
B. Conidiophores fuscous, terminating in hyaline, somewhat swollen, distal cells that bear the conidia singly on stout sterigmae.....35. *acrophalarium* Moore
B. Conidiophores brownish hyaline throughout, conidia borne as continuations of the terminal cells.....13. *limpidum* Morgan
31′. [*H. recurvum* (Petch) Linder]
31′. Conidiophores not producing crystalloid masses.....C
C. Conidiophores long, slender, about 4 μ thick by up to 600 μ long, usually once-branched ½–⅓ the distance from the base; conidia profuse, acropleurogenous, pale fuscous, (7–)8–12(–16)-septate, 15.5–19.5 μ diam., filaments 5.5–8 μ thick, coiled 1½–1¾ times.....31. *taenia* Moore
C. Conidiophores shorter, stouter, 125–130 × 5–6 μ, unbranched; conidia few, acropleurogenous, hyaline to very pale yellowish, (7–)11–13-septate, 18–20 μ diam., filaments 6.5–8 μ thick, coiled 1½ times.....36. *interveniens* Talbot (7)

KEY TO THE SPECIES OF HELICOSPORIUM: ADDENDA

12′. Conidia borne on bladder-like projections.....A
12′. Conidia not borne on bladder-like projections.....13′
13′. Filaments 1 μ thick, conidia diameter 12.5–15 μ; conidiophores sparsely branching below.....14
13′. Filaments 1.5–2.5 μ thick, conidial diam. 15–30 μ.....B
14. Conidiophores clearly septate, mostly simple, not anastomosing above, 108–250 × 3.5–4.5 μ; conidia 12.5–14.5 μ diam.....13. *griseum* (Bon.) Saccardo
14. Conidiophores indistinctly septate, elongate, slender, sparsely anastomosing above, up to 580 μ long by 1.5–4 μ thick; conidia 10–15 μ diam.....14. *pallidum* Cesati
A. Conidia 6–9 μ diam., filaments 0.75–1.5 μ thick, coiled 1–2 times; conidiophores very slightly flexuous above, 75–200 μ high, becoming branched and decumbent.....11. *decumbens* Linder
A. Conidia 13–18 μ diam., filaments 1–2 μ thick, coiled up to 5 times; conidiophores tapering-flexuous and frequently branching above, 350–400 μ high, remaining erect.....18. *Neesii* Moore
B. Conidiophores not anastomosing; conidia coiled 3–4 times, multiseptate, 15–18 μ diam.....12. *phragmitis* Höhnel
B. Conidiophores often anastomosing above; conidia coiled 1½–2½ times, about 15-septate, diam. 19–24(–27) μ.....19. *ramosum* Talbot (7)
15′. Filaments 5.5–8 μ thick.....C
15′. Filaments 8–10 μ thick.....16
C. Conidiophores granulate-roughened by crystal-like deposits, bearing the conidia on stout teeth 4 × 5–7 μ; filaments fuscous to dilute fuscous, coiled 3–5 times, often in 3 planes.....15. *Elinorae* Linder
C. Conidiophores without crystal deposits, bearing the conidia on stout teeth 3–4 × 5–5.5(–10) μ; filaments hyaline, strongly curved to once spiraled, brittle; colonies producing long, sterile, tapering setae.....20. *pannosum* (Berk. & Curt.) Moore

KEY TO THE SPECIES OF HELICOMYCES: ADDENDUM

4. Sterile mycelium and conidiophores fuscous or dilute fuscous.....A
4. Sterile mycelium and conidiophores mostly hyaline.....6
A. Conidia 12.5–19 μ diam., filaments about 2 μ thick; conidiophores stout, branched or unbranched, up to 40 × 5–6 μ.....8. *lilliputeus* Moore
A. Conidia (23–)35–62 μ diam., filaments 2.5–4.5 μ thick.....5

KEY TO THE SPECIES OF XENOSPORELLA: ADDENDUM

1′. Conidia 15–30 μ in diameter.....2
1′. Conidia 30–55 μ in diameter.....A
A. Mature conidia deep brown to opaque brownish-black, somewhat spirally coiled and without central cells, filaments composed of 28–65 cells, medially 20–25 μ thick, coiled diam. 37–57 μ; conidiophores 15–25 μ high.....1. *Thaxteri* Linder
A. Mature conidia dilutely colored, coiled ¾–1¼ times around 1–5 deep-fuscous central cells, each 4–6.7 μ diam., filaments composed of 26–34 cells, medially 14.6–18.5 μ thick, coiled diam. 33–44 μ; conidiophores up to 10 μ high.....5. *rosea* Talbot (7)

This paper was initiated at the Quartermaster Research and Development Center, but amplified and brought to completion under the direction of Prof. I. M. Lamb of the Farlow Herbarium.

DEPARTMENT OF BIOLOGY
HARVARD UNIVERSITY
CAMBRIDGE, MASSACHUSETTS

LITERATURE CITED

1. **Boedijn, K. B.** 1942. *Circinoconis*, a new genus of Dematiaceae (Fungi Imperfectae). 150th Anniv. Vol. R. Bot. Gard. Calcutta: 209–211.
2. **Berkeley, M. J.** 1875. Notices of North American fungi. Grevillea **3**: 105.
3. **Glen-Bott, Janet I.** 1955. On *Helicodendron tubulosum* and some similar species. Brit. Myc. Soc. Trans. **38**: 17–30.
4. **Höhnel, F. von.** 1910. Fragmente zur Mycologie 566. Sitzungsb. k. k. Akad. Wien, Math.-Nat. Kl. **119**: 617–679.
5. **Hughes, S. J.** 1953. Conidiophores, conidia, and classification. Can. Jour. Bot. **31**: 577–659.
6. **Moore, Royall T.** 1955. Index to the Helicosporae. Mycologia **47**: 90–103.
7. **Talbot, P. H. B.** 1956. New and interesting records of South African fungi. Part II. Bothalia **6**: 489–500.

HYPOXYLON PUNCTULATUM AND ITS CONIDIAL STAGE ON DEAD OAK TREES AND IN CULTURE [1]

H. L. Barnett

(with 17 figures)

Among the fungi that appear frequently on standing dead oak-wilt trees in West Virginia are several species of *Hypoxylon*. The most common species, judging from field observations and isolations from wood of dead oaks, is *H. punctulatum* (Berk. & Rav.) Cke., which produces its black, shining, effused stromata within the bark of trunks and branches. The stromata are exposed only after the outer layer of bark has been thrown off.

Closely associated with the fertile stromata of *H. punctulatum* and arising from about the same bark region, one frequently finds the darkened remains of a conidial fungus in the cracks of the bark. Collections of younger stages showed abundant production of light gray globose heads of conidia, which resemble young heads of *Aspergillus*. Specimens of both natural material and pure cultures were examined by Dr. S. J. Hughes, who confirmed the identification of this fungus as belonging to the genus *Basidiobotrys*.

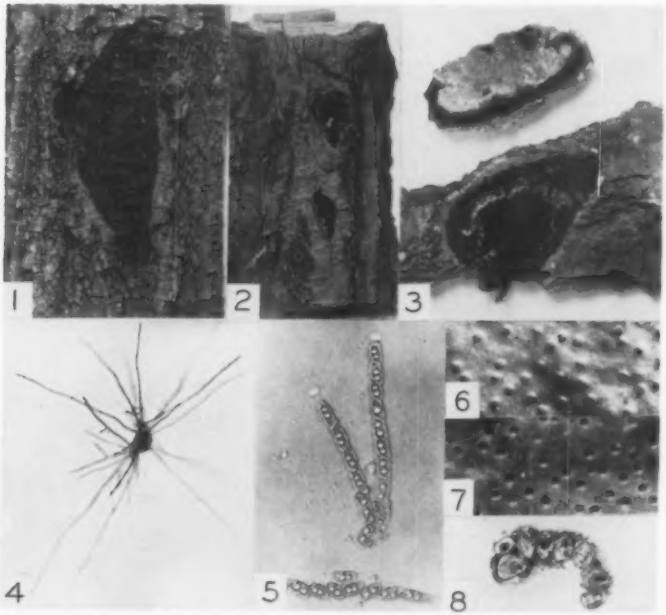
Two unsolved problems relating to *H. punctulatum* have prompted the investigations reported herein. These were first, the method by which the bark covering of the stroma is thrown off and second, the possible relationship of the conidial fungus to *H. punctulatum*. This paper reports the results of these investigations.

THE HYPOXYLON STAGE

The stromata occur more commonly on black and red oaks than on white oaks in West Virginia. Growth and development of the fungus in the tree occurs rapidly. One black oak tree 8 inches in diameter bore numerous small exposed stromata and others just breaking through the bark in January 1957. This tree had shown symptoms of oak wilt in June 1956 and had been girdled at that time.

[1] Published with approval of the Director of the West Virginia Agricultural Experiment Station as Scientific Paper No. 548.

The perithecial stromata vary in size from about one inch in diameter to about two inches in width and several inches long. The flat stromata in which the perithecia are completely imbedded develop in the region between the inner and outer bark and may be as deep as 6 mm (FIG. 1). As the perithecia approach maturity the bark covering breaks loose at the edge of the stroma and is cast off forcibly (FIGS. 2, 3). The method



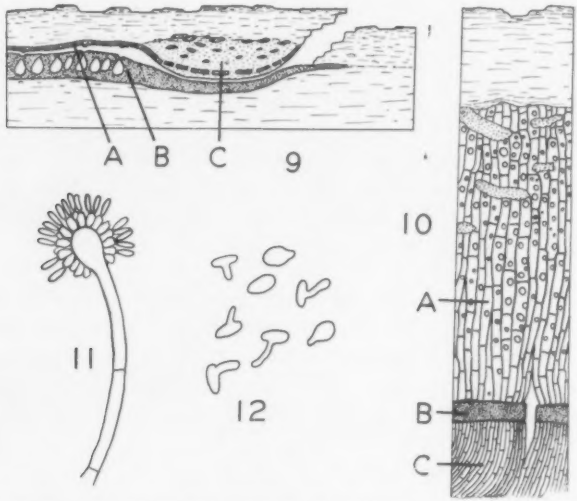
FIGS. 1–8. *Hypoxylon punctulatum*. 1. Mature stroma after bark covering has been thrown off, × ½. 2. Partially exposed stromata, × ½. 3. Exposed small entostroma (below) and bark covering inverted (above) to show surface of ectostroma and gelatinous ridge at the edge, × ½. 4. Single ascospore germinating by several germ tubes, × 200. 5. Asci and ascospores, × 400. 6. Papillate surface of ectostroma, × 10. 7. Pitted surface of entostroma, × 10. 8. Swollen antolyzed ascospores from a single ascus, × 200.

by which sufficient force was created to crack the thick bark covering was unknown until several young fresh specimens were collected during early winter of 1956.

The fresh ectostroma, which is attached to the outer bark covering, is a thin gray layer except near its edge, where a ridge of gelatinous

tissue is formed (FIG. 3). Apparently this tissue imbibes water, swells and pushes against the edge of the entostroma with sufficient pressure to break the bark. The gelatinous ridge is approximately 2 to 3 mm thick when moist and 1 mm thick when dry. The structure and relative positions of the ectostroma, entostroma and gelatinous ridge are shown diagrammatically in FIG. 9.

Early stages prior to the rupture of the bark have not been observed, but it appears that the gelatinous ridge arises in the region of the ecto-



FIGS. 9–12. *Hypoxylon punctulatum*. 9. Diagram of a section through the edge of a stroma and bark. A, ectostroma; B, entostroma; C, gelatinous tissue, × 3. 10. Diagram of a section through outer bark and gelatinous tissue. A, outer palisade; B, broken ectostroma; C, inner palisade, × 25. 11. Conidiophore bearing sporogenous cells and conidia, from fungus pillar in bark cavity, × 300. 12. Germinating conidia after 2 days on agar, × 500.

stroma, for pieces of the latter are imbedded in the gelatinous tissue when it has reached full size. This tissue is made up of three distinct layers: The outer (toward the outside of the tree) palisade, the broken ectostroma and the inner palisade (FIG. 10). The outer layer makes up nearly three-fourths of the thickness and is composed of hyphae arranged perpendicularly to the bark surface. The elongated cylindrical cells are tightly compressed and many of them contain oil globules. The thin ectostroma, being a rigid tissue, is broken by the swelling of the

outer palisade and is pushed inward. It is compact and carbonaceous and its structure is difficult to see. The inner palisade is composed of tightly packed slender hyphae which push against the entostroma as the gelatinous ridge swells.

The freshly exposed surface of the ectostroma bears numerous papillae (FIG. 6), while the surface of the entostroma is punctulate (FIG. 7). Before separation, the papillae of the ectostroma fitted into the shallow pits of the entostroma. Ostioles of perithecia are located directly beneath the pits.

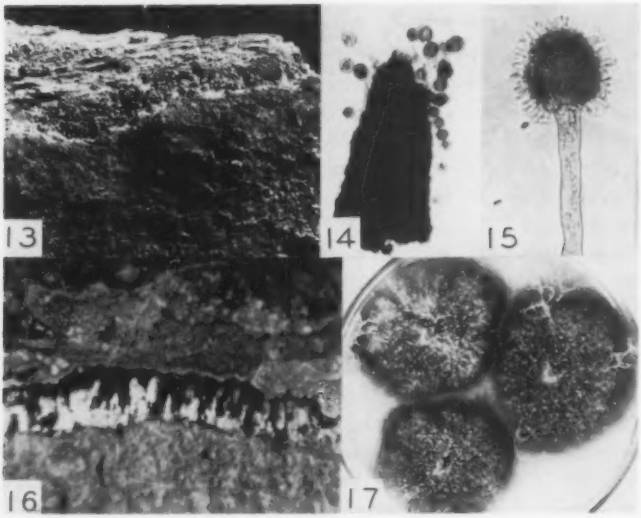
Most of the entostromata collected contained immature asci and ascospores but some of the larger ones collected during winter contained mature ascospores. Eighteen small stromata from 8 different trees were surface-sterilized by flaming alcohol and bits of the stromal tissue placed on agar media. A total of over 60 cultures were obtained. All stromata yielded the typical *Basidiobotrys* conidial stage and no other fungus, except some common contaminants. Wounded stromata left in the moist chamber for a few days produced the *Basidiobotrys* conidial heads only on the wounded surface.

Portions of six stromata containing mature asci were surface-sterilized and chips cut away to expose the perithecia, which were lifted out and placed in sterile distilled water. The perithecia were then crushed to release the ascospores and the water, containing ascospores, asci and bits of perithecia, was poured over an agar medium containing 5 gm glucose and 1 gm yeast extract per liter, and placed at 20° and 25° C. Asci and ascospores are shown in FIG. 5.

Germination of ascospores under these conditions was extremely poor. Some of the ascospores, even while in the ascus, began to swell within 24 hours and increased in size until reaching a diameter of 30 to 40 μ by the end of three days. Most of the swollen spores showed distinct autolysis (FIG. 8). Much of the granular contents oozed out and collected around the spores. The swollen spores that did not undergo autolysis germinated after 3 or 4 days at 25° C by means of several germ tubes produced almost simultaneously (FIG. 4). The rate of autolysis and germination was slower but the percentage of germination was greater at 20° than at 25° C. A total of approximately 40 isolations were made from germinating ascospores, including 5 single ascospore cultures. All of these produced typical conidial heads of *Basidiobotrys* within a few days (FIG. 15). These results are considered as conclusive evidence that the conidial stage of *Hypoxylon punctulatum* belongs to the genus *Basidiobotrys*.

THE BASIDIOBOTRYS STAGE

The first sign of the *Basidiobotrys* stage on a dead oak tree is the cracking of the outer bark, revealing a narrow tangential cavity which may be as deep as 6 mm in the bark. The bark covering remains in place but it can be pulled off, exposing the light gray sporulating area, which may be 1 to 2 inches wide and several inches in length (FIG. 13). This stage usually appears before the *Hypoxylon* stromata are formed, but the perithecial stromata have been observed on trees on which no



FIGS. 13–17. *Hypoxylon punctulatum*. 13. Bark of oak with outer layer partially removed to show conidial fruiting layer, × ¾. 14. Upper portion of fungus pillar bearing conidiophores and sporogenous cells (conidia have been shed), × 4. 15. Conidial head from culture, × 100. 16. Section through bark showing tangential cavity and fungus pillars, × 500. 17. Culture originating from ascospores growing on glucose-yeast extract agar.

conidia were found. Often the darkened remains of the conidial fructification can be seen after the perfect stage is produced. Both stages originate in the area between the outer and inner bark and are often in close proximity, but the conidial heads are not formed normally on the surface of the perithecial stromata.

The early stages in the development of the conidial fructification before the bark is cracked, have not been observed. The mature struc-

ture shows an unusual method of producing spores in a cave-like area in the bark. Apparently the outer bark is pushed upward by means of numerous pillars of compact fungus tissue arising from a thin stroma-like basal layer (FIG. 16). The pillars reach a length of 1 to 1.5 mm, the longer ones extending the entire width of the cavity. The surface of the pillars and the floor of the cavity is covered with conidiophores and globose beads of conidia (FIGS. 11, 14). Insects have been observed frequently in these cavities and it is probable that some of them use the fungus as food and possibly act as vectors.

A low percentage of conidia from culture germinated on a glucose-yeast extract agar at pH 5.0 at 25° C. These formed short germ tubes that soon ceased to grow (FIG. 12). Conidia from bark failed to germinate in a limited number of trials. New cultures were started easily from bits of mycelium.

Cultures from all sources produced the typical *Basidiobotrys* stage on agar but were somewhat variable in appearance under the same conditions. Under most conditions the conidial heads were spherical, as they are on the bark, but sometimes in culture they were more elongate and the sporogenous cells more loosely arranged. Growth of the mycelium was more rapid on agar containing 20 gm glucose per liter than on that containing 5 gm. Under some conditions not fully understood, portions of the mycelium grow more rapidly and become feathery in appearance, due to numerous, slender lateral branches on the main hyphae (FIG. 17). It is believed that these differences in appearance are due to nutrition, environment, or pH, rather than to genetic changes. Older cultures in test tubes usually produce small, black, carbonaceous masses of tissue similar in structure to stromal tissue. The agar medium usually becomes stained with a dark greenish or brownish pigment. Conidial heads are usually abundant. Much more physiological work must be done before the effects of the various factors on growth and sporulation can be determined.

DISCUSSION

The frequent isolation of the *Basidiobotrys* conidial stage in pure culture from wood beneath the perithecial stromata of *Hypoxylon punctulatum* and the frequent development of the perithecial stromata in close proximity to the remains of the *Basidiobotrys* fructification within the bark of dead oak trees suggested that these two stages belonged to the same species. Subsequent consistent isolation of *Basidiobotrys* in pure culture from bits of perithecial stromata and from germinating ascospores of *H. punctulatum* confirmed this hypothesis.

This species of *Hypoxylon* has been reported from the Eastern states on a number of deciduous trees, but the first record of *Basidiobotrys* from West Virginia was based on isolations made in 1952. Increasingly frequent isolations since that time may indicate that the fungus is becoming more abundant on oak trees in this region.

The conidial stage of *H. punctulatum* is different from that of most other species of *Hypoxylon*, which usually produce powdery conidia on the surface of the exposed ectostroma (Miller, **1928**). However, *H. tinctor*, which has a developmental pattern similar to that of *H. punctulatum* (Miller, **1928**), produces in culture elongated club-like conidial heads that would fall into the imperfect genus *Basidiobotrys*. These heads are somewhat larger and the sporogenous cells are usually less compact than those of *H. punctulatum* (unpublished).

Both the ascospores and conidia of *H. punctulatum* germinate slowly and with difficulty under the conditions used. One may conclude that special nutritional or environmental conditions are necessary for better germination, but this problem has not been investigated.

The production of a gelatinous ridge of tissue between the ectostroma and entostroma appears to be a special adaptation for throwing off the outer bark and exposing the perithecial layer. This general mechanism, involving the production of specialized tissues and resulting in the cracking of this bark covering and exposure of sporulating surfaces, is probably common to many bark and wood inhabiting fungi. One well known example is *Endoconidiophora fagacearum*, which produces a pressure cushion near the center of a sporulating mat (Leach *et al.*, **1952**). Similarly, Wehmeyer (**1926**) has described the shedding of the bark covering of *Diatrype stigma* as being accomplished by swollen fungus cells of a "palisade-like pseudoparenchymateous tissue" in the bark.

The method by which *H. punctulatum* produces its conidial heads on pillars of fungus tissue in cave-like cracks in the bark is also of interest. In the tree this stage is commonly attacked by fungus-feeding insects which possibly play a role in dissemination. This method is similar to that described by Gregory and Waller (**1951**) for *Cryptostroma corticale* on *Acer pseudoplatanus*. Further studies in the stromatic, wood-inhabiting fungi are needed before their conidial stages can be understood and recognized in culture.

SUMMARY

Evidence is presented leading to the conclusion that the *Basidiobotrys* isolated frequently from oak wilt trees in West Virginia is the conidial stage of *Hypoxylon punctulatum*.

The specialized mechanism by which the bark covering of the perithecial stroma is thrown off involves the formation of a ridge of gelatinous tissue which exerts pressure as it swells and breaks the bark at the edge of the stroma. In the conidial stage a cavity is formed in the bark by the pressure created by the fungus pillars on which the conidial heads are formed.

The ascospores and conidia germinate poorly on agar and probably require special conditions for normal germination.

ACKNOWLEDGMENTS

The author is indebted to Dr. R. P. True, Mr. William Gillespie and Mr. A. L. Shigo for collecting the material used in this study, and to Dr. J. H. Miller for identifying the species of *Hypoxylon*.

WEST VIRGINIA UNIVERSITY
MORGANTOWN, WEST VIRGINIA

LITERATURE CITED

Gregory, P. H. and S. Waller. 1951. *Cryptostroma corticale* and sooty bark disease of sycamore (*Acer pseudoplatanus*). Trans. Brit. Mycol. Soc. **34**: 579–597.

Leach, J. G., R. P. True and C. K. Dorsey. 1952. A mechanism for liberation of spores from beneath the bark and for diploidization in *Chalara quercina*. Phytopath. **42**: 537–539.

Miller, J. H. 1928. Biologic studies in the Sphaeriales II. Mycologia **20**: 305–339.

Wehmeyer, L. E. 1926. Further cultural life histories of the stromatic Sphaeriales. Amer. Jour. Bot. **13**: 231–246.

RHODA WILLIAMS BENHAM
1894–1957

MARGARITA SILVA AND ELIZABETH L. HAZEN

(WITH PORTRAIT)

"Something very profound has changed in our American horizon with the passing away of this pioneer in medical mycology. . . ." Thus wrote one of Dr. Benham's British colleagues upon hearing the news of her untimely death. That Dr. Benham was indeed a pioneer in the study of pathogenic fungi, and in the founding with the late Dr. J. Gardner Hopkins of the first laboratory for research in, and teaching of, Medical Mycology in this country, is well known by her many associates, students, and fellow mycologists throughout the world.

Rhoda Williams Benham was born on December 5, 1894 in Cedarhurst, New York. Her family, although living for some time in Long Island, had a long New England ancestry and formed a closely knit group, the warmth and affection of which had a profound influence on Dr. Benham's life. Cedarhurst was always her home, and even during the years when she maintained an apartment in New York, it was in Cedarhurst that she spent her week ends and vacations, and entertained many of her friends and visiting mycologists.

Having completed her primary and secondary education in Cedarhurst, she attended Barnard College of Columbia University, and in 1917 received a B.A. degree, majoring in Botany. From 1918 to 1925 Dr. Benham remained affiliated with the Botany Department at Barnard, as a graduate student and teaching assistant in Botany. During that period she received the stimulating guidance of four prominent botanists in the Columbia faculty, three of them mycologists of international renown: Prof. Herbert M. Richards, Prof. R. A. Harper, Dr. B. O. Dodge, and Dr. Tracy Hazen. The influence of these great teachers profoundly affected Dr. Benham in the selection of a career.

Dr. Benham received her master's degree in Botany at Columbia in 1919 and under Prof. Richards began work toward the degree of Doctor of Philosophy. For her thesis, she started a study of the metal nutrition of *Aspergillus niger*.

About this time Dr. J. Gardner Hopkins became Professor of Dermatology at the College of Physicians and Surgeons of Columbia Uni-

RHODA W. BENHAM

versity. Dr. Hopkins, recognizing the importance of fungous diseases in dermatology, decided that his department should have a laboratory for the study of pathogenic fungi. In looking for a suitably trained assistant, he sought the advice of his long-time friend, Bernard O.

Dodge, who recommended a promising young student of mycology at Barnard, Miss Rhoda Benham. Dr. Hopkins was so favorably impressed with this young mycologist that he invited her to join his staff as an Assistant in Dermatology and thus began the long and productive association which decisively shaped Dr. Benham's career.

Starting work on pathogenic fungi meant an interruption of Dr. Benham's work at Barnard, but this new interest soon provided material for another thesis: "Certain Monilias Parasitic on Man, their Identification by Morphology and by Agglutination," for her Ph.D. in Botany in 1931.

This particular work, now considered a classic, was first in the application of immunologic principles as a taxonomic tool in the study of pathogenic fungi. The numerous species of *Candida,* known in those days as the "medical monilias," presented a very confusing problem. Her painstaking comparison of strains designated as *Monilia psilosis, Endomyces albicans* and even *Saccharomyces* and *Blastomyces,* demonstrated that the majority of isolates of fermenting yeast-like fungi from human lesions were identical and comprised a single species, *Candida* (*Monilia*) *albicans.* Furthermore, she described criteria by which this species could be clearly differentiated from others such as *C. krusei, C. parapsilosis* and *C. tropicalis.* For the study of their microscopic morphology she recommended corn meal infusion agar, a culture medium that, prepared by her method, remains unsurpassed for the rapid induction of the diagnostic chlamydospores of *C. albicans.* By developing specific antisera in rabbits, she demonstrated antigenic differences and similarities among the various species in this group. This publication and subsequent ones on the genus *Candida,* as well as a similar treatment of the genus *Cryptococcus,* justly established Dr. Benham as an authority on the yeast-like fungi pathogenic to man.

Her studies, however, were by no means confined to these two genera. Her publications on *Phoma condiogena, Sporotrichum schenkii, Pityrosporum ovale, Allescheria boydii,* the genus *Beauveria,* and last but not least, the dermatophytes, attest to the breadth of her interest in the fungi.

Dr. Benham was primarily a taxonomist, and for her studies she frequently made use not only of morphology, but also of biochemical behavior, including nutrition, and of antigenic resemblances, and virulence.

Dr. Benham's interest in the nutrition of fungi was aroused by her finding that *Pityrosporum ovale* required oleic acid for growth. She later studied its vitamin nutrition and thus introduced in her laboratory

the study of the nutrition of fungi, a field in which several other investigators were interested at the time. Her publications on the nutrition of dermatophytes, many of them in collaboration with her students and associates, have significantly enlarged our knowledge of this field.

In addition to Dr. Benham's production as an investigator, another significant advantage obtained from the establishment of this laboratory was that it served as a nucleus for the training of medical mycologists. Supported initially by a generous grant from the Rockefeller Foundation and with Dr. Hopkins and Dr. Dodge as recruiters, the laboratory, from the time of its inception, nurtured many young scholars who later attained prominence. In the years to follow, the reputation of the laboratory became widely known, and continued to attract students from near and far.

Among the many who worked or studied in this laboratory the following are included: Beatrice M. Kesten, Chester W. Emmons, Mary E. Hopper, Arturo L. Carrión, Edward De Lamater, Otis Jillson, V. Medd Henington, Lucille Georg, Floriante Bocobo, Roland Riddell, Jose Miranda, Milton Huppert, Edith Schnall, and many others too numerous to be listed.

In 1935, Dr. Benham, with the aid of Drs. Hopkins and Dodge, organized a course in Medical Mycology as one of the post-graduate courses offered by the College of Physicians and Surgeons at Columbia University. This course, intended for graduate students, and offered jointly by the Departments of Microbiology and Dermatology in the Medical School, was the first course in medical mycology in the country and has served as a stimulus for the establishment of similar courses in other academic institutions.

Dr. Benham was a dedicated teacher. She spent long and tedious hours in preparation of her lectures and in planning the laboratory exercises. Her first-hand experience with fungous pathogens, and her thorough and critical knowledge of the literature, were reflected in her lectures, which abounded in valuable, well-organized information. She made ample use of lantern slides; they included not only color transparencies of clinical lesions, histopathology, and gross and microscopic morphology of fungi in cultures, but also taxonomic keys and charts indicating phylogenetic relationship of the various fungous groups under discussion. Many of these slides were prepared as a result of her own observations and conclusions. This graphic presentation greatly increased the impact of her lectures. Her laboratory manual helped show the students how to examine living cultures, many of them prepared by the students themselves; it also was a guide to demonstrations of

preserved material and permanent microscopic slides which illustrated noteworthy features of the parasitic and *in vitro* aspects of the fungi.

Throughout the years, Dr. Benham was able to assemble a large culture collection, of immeasurable value, not only for the research and teaching conducted in her laboratory, but also to supply the needs of many investigators in other institutions. She was always available for consultation in problems of isolation and identification of pathogenic fungi, giving advice freely, or undertaking the study of specimens, as the case required. This was done, not only for members of her own medical community, but also for many others who sought her advice.

Photography was Dr. Benham's hobby. She found the same joy in photographing her fungi at work, as she did in photographing flowers, trees and members of her family and friends at play. Unlike so many who seek pleasure and relaxation in activities unrelated to their daily work, her cameras always accompanied her on her holiday week-ends and vacations. The showing of each new set of color transparencies was often the excuse for informal gatherings which gave great pleasure to many of her friends. Some of her pictures of trees, enlarged from her black and white negatives, won honorable mention at the annual art exhibits of the Medical Center Personnel. Her collection of photographs of patients, of fungous cultures (both gross and microscopic), and of histologic sections from infected tissues, illustrating both deep and superficial mycoses, is another legacy left by her to this Department. Her inclination to photography enabled her to prepare excellent exhibits presented, with her associates, at several successive annual conventions of the American Medical Association, and one convention each of the American Public Health Association, the Society of American Bacteriologists and at the Ninth International Dermatological Congress held in Budapest in 1935. Many of these exhibits were awarded gold medals or certificates of merit for their excellence.

Dr. Benham was honored by being elected several times to serve as a member of various committees for the consideration of problems on fungous infections. She was one of the editors of the chapter on "Pathogenic Fungi" for the fourth edition of "Diagnostic Procedures and Reagents" of the American Public Health Association. She also served on the committee on Medical Mycology charged with preparation of proposals regarding nomenclature for the International Botanical Congress in Stockholm in 1950. At this Congress her proposal was approved for the conservation of the generic name *Candida* for the medical monilias. She was also the author of the chapter "Pathogenic Fungi" in Gay *et al.*, "Agents of Disease and Host Resistance." At the

invitation of Dr. Hilleboe, Commissioner of Health for the State of New York, she served as consultant in mycology to his department from 1948 through 1953.

Dr. Benham held membership in many scientific societies and organizations: The Society of the Sigma Xi, the Harvey Society, the Microbiological Section of the New York Academy of Medicine, the Society of Investigative Dermatology, the Society of American Bacteriologists, the New York Academy of Sciences, the American Association for the Advancement of Science, and last but not least the Mycological Society of America. She was one of the original editors of the international publication *Mycopathologia*.

In 1948 Dr. Benham's health began to fail, a heart attack required hospitalization and absence from her laboratory for nearly a year. She recovered sufficiently to resume active duty for several more years. During that time her work on the genus *Beauveria*, and additional work on the cryptococci and dermatophytes were completed. In the summer of 1955 her health failed again and she was unable to return to the laboratory. She continued writing, however, completing the manuscripts for her last two papers. After one and one-half years spent in her home in Cedarhurst, she died on January 17, 1957. Dr. Benham was an Episcopalian and was buried in her family parish in Hewlett, Long Island, New York. Her ill health had forced her premature retirement as Associate Professor in the Department of Dermatology at Columbia.

Perhaps the greatest honor a person could receive, the recognition of friends and former students, was granted to Dr. Benham. This was expressed in the form of an album of letters and photographs contributed by over seventy of her friends, students and associates, and presented to her on her sixty-second birthday. This album gave Dr. Benham great joy and the satisfaction of knowing that, though retired, she had not been forgotten.

COLLEGE OF PHYSICIANS AND SURGEONS
NEW YORK 32, N. Y.
AND
NEW YORK STATE DEPT. OF HEALTH
ALBANY 1, N. Y.

PUBLICATIONS OF RHODA W. BENHAM

1. Monilia infections of the hands and feet. New York State Jour. Med. **29**: 793–800. 1929. (With J. G. Hopkins.)
2. Asthma due to a fungus, *Alternaria*. Jour. A. M. A. **94**: 6–10. 1930. (With J. G. Hopkins and B. M. Kesten.)

3. Sensitization to saprophytic fungi in a case of eczema. Proc. Soc. Exp. Biol. Med. **27**: 342–344. 1930. (With J. G. Hopkins and B. M. Kesten.)
4. Certain monilias parasitic on man; their identification by morphology and by agglutination. Jour. Infect. Dis. **49**: 183–215. 1931.
5. *Phoma condiogena*, an excitant in asthma: some observations on the development and cultural characteristics. Bull. Torrey Bot. Club **58**: 203–214. 1931.
6. Monilias, yeasts and cryptococci; their pathogenicity, classification and identification. Am. Jour. Pub. Health **22**: 502–504. 1932.
7. Sporotrichosis in New York State. New York State Jour. Med. **32**: 595–601. 1932. (With J. G. Hopkins.)
8. Sporotrichosis: its transmission to plants and animals. Jour. Infect. Dis. **50**: 437–458. 1932. (With B. M. Kesten.)
9. Fungus infections of the skin and its appendages occurring in Puerto Rico; a clinical and mycologic study. Arch. Derm. Syph. **25**: 1046–1057. 1932. (With B. M. Kesten, B. K. Ashford, C. W. Emmons and M. C. Moss.)
10. Vegetable parasites that attack both plant and human life. Jour. New York Bot. Garden **33**: 149–153. 1932.
11. Yeast-like fungi found on the skin and in the intestines of normal subjects; a survey of 100 persons. Arch. Derm. Syph. **28**: 532–543. 1933. (With A. McH. Hopkins.)
12. The fungi of blastomycosis and coccidioidal granuloma. Arch. Derm. Syph. **30**: 385–400. 1934.
13. The terminology of the cryptococci with a note on *Cryptococcus mollis*. Mycologia **27**: 496–502. 1935.
14. Cryptococci—their identification by morphology and by serology. Jour. Infect. Dis. **57**: 255–274. 1935.
15. Pathogenic Fungi; Chapter 45 in "Agents of Disease & Host Resistance," Gay *et al.*, Ch. C. Thomas, Springfield. 1935.
16. Experimental studies with the dermatophytes. 1. Primary disease in laboratory animals. Jour. Invest. Dermat. **1**: 451–467. 1938. (With E. D. DeLamater.)
17. Experimental studies with the dermatophytes. 2. Immunity and hypersensitivity produced in laboratory animals. Jour. Invest. Dermat. **1**: 469–488. 1938. (With E. D. DeLamater.)
18. The fungi causing deep-seated infections: their diagnostic characteristics and classification. Proc. 6th Pacific Sci. Congress **5**: 863–872. 1939.
19. The cultural characteristics of *Pityrosporum ovale*—a lipophylic fungus. Jour. Invest. Dermat. **2**: 187–203. 1939.
20. Cultural characteristics of *Pityrosporum ovale*—a lipophylic fungus. Nutrient and growth requirements. Proc. Soc. Exp. Biol. Med. **46**: 176–178. 1941.
21. The nutrient and growth requirements of *Pityrosporum ovale*—a lipophylic fungus. (Abstract) Torreya **41**: 68–69. 1941.
22. Laboratory procedures in the diagnosis of fungus diseases. Proc. New York State Assoc. Pub. Health Labs. **24**: 69–82. 1944.
23. *Pityrosporum ovale*—a lipophylic fungus. Thiamin and oxaloacetic acid as growth factors. Proc. Soc. Exp. Biol. Med. **58**: 199–201. 1945.
24. Biology of *Pityrosporum ovale*. Chapter 4 in Nickerson's "Biology of Pathogenic Fungi," Ann. Cryptogam. Pytopathol. VI, Chronica Botanica Pub. Co., Waltham, Mass. 1947.

25. *Allescheria boydii*, causative agent in a case of meningitis. Jour. Invest. Dermat. **10**: 99–110. 1948. (With L. K. Georg.)
26. Effect of nutrition on growth and morphology of the dermatophytes. 1. Development of macroconidia in *Trichophyton rubrum*. Mycologia **40**: 232–240. 1948.
27. Pigment production in the differentiation of *Trichophyton mentagrophytes* and *Trichophyton rubrum*. Mycologia **41**: 291–302. 1949. (With F. C. Bocobo.)
28. Cryptococcosis and blastomycosis. Ann. New York Acad. Sci. **50**: 1299–1314. 1950.
29. The Laboratory of Medical Mycology. The Biopsy **2**: 1–2. 1950.
30. Nutritional studies of the dermatophytes with special reference to *Trichophyton megnini* Blanchard 1896 and *Trichophyton gallinae* (Megnin 1881), comb. nov. Jour. Invest. Dermat. **18**: 453–472. 1952. (With M. Silva.)
31. An unusual finding in *Epidermophyton floccosum*. Jour. Invest. Dermat. **19**: 315–317. 1952. (With P. McCormack.)
32. Nutritional studies of the dermatophytes—effect on growth and morphology, with special reference to the production of macroconidia. Trans. New York Acad. Sci. **15**: 102–106. 1953.
33. Maduromycosis of the central nervous system. Jour. Neuropath. & Exp. Neurol. **12**: 158–168. 1953. (With S. M. Aronson and A. Wolf.)
34. The genus *Beauveria*, morphological and taxonomical studies of several species and of two strains isolated from wharf-piling borers. Mycologia **45**: 727–746. 1953. (With J. L. Miranda.)
35. Chemical analysis of the capsular substance of *Cryptococcus neoformans*. Jour. Invest. Dermat. **22**: 279–283. 1954. (With J. M. Einbinder and C. T. Nelson.)
36. Nutritional studies of the dermatophytes with special reference to the red-pigment producing varieties of *Trichophyton mentagrophytes*. Jour. Invest. Dermat. **22**: 285–294. 1954. (With M. Silva.)
37. Treatment of onychomyocis due to *Trichophyton rubrum*. A. M. A. Arch. Dermat. **71**: 52–55. 1955. (With B. M. Kesten and M. Silva.)
38. The genus *Cryptococcus:* the present status and criteria for the identification of species. Trans. New York Acad. Sci. **17**: 418–429. 1955.
39. *Cryptococcus neoformans:* "an ascomycete." Proc. Soc. Exp. Biol. Med. **89** 243–245. 1955.
40. *Trichophyton rubrum* infections: a clinical, mycologic and experimental study. Jour. Invest. Dermat. **25**: 311–328. 1955. (With M. Silva and B. M. Kesten.)
41. The genus *Cryptococcus*. Bact. Reviews **20**: 189–200. 1956.
42. Species of *Candida* most frequently isolated from man: methods and criteria for their identification. Jour. Chronic Dis. **5**: 460–472. 1957.
43. The chlamydospores of *Candida albicans:* comparison of three media for their induction. Jour. Lab. & Clin. Med. 1957. (in press—posthumously) (With J. D. Pollack.)

preserved material and permanent microscopic slides which illustrated noteworthy features of the parasitic and *in vitro* aspects of the fungi.

Throughout the years, Dr. Benham was able to assemble a large culture collection, of immeasurable value, not only for the research and teaching conducted in her laboratory, but also to supply the needs of many investigators in other institutions. She was always available for consultation in problems of isolation and identification of pathogenic fungi, giving advice freely, or undertaking the study of specimens, as the case required. This was done, not only for members of her own medical community, but also for many others who sought her advice.

Photography was Dr. Benham's hobby. She found the same joy in photographing her fungi at work, as she did in photographing flowers, trees and members of her family and friends at play. Unlike so many who seek pleasure and relaxation in activities unrelated to their daily work, her cameras always accompanied her on her holiday week-ends and vacations. The showing of each new set of color transparencies was often the excuse for informal gatherings which gave great pleasure to many of her friends. Some of her pictures of trees, enlarged from her black and white negatives, won honorable mention at the annual art exhibits of the Medical Center Personnel. Her collection of photographs of patients, of fungous cultures (both gross and microscopic), and of histologic sections from infected tissues, illustrating both deep and superficial mycoses, is another legacy left by her to this Department. Her inclination to photography enabled her to prepare excellent exhibits presented, with her associates, at several successive annual conventions of the American Medical Association, and one convention each of the American Public Health Association, the Society of American Bacteriologists and at the Ninth International Dermatological Congress held in Budapest in 1935. Many of these exhibits were awarded gold medals or certificates of merit for their excellence.

Dr. Benham was honored by being elected several times to serve as a member of various committees for the consideration of problems on fungous infections. She was one of the editors of the chapter on "Pathogenic Fungi" for the fourth edition of "Diagnostic Procedures and Reagents" of the American Public Health Association. She also served on the committee on Medical Mycology charged with preparation of proposals regarding nomenclature for the International Botanical Congress in Stockholm in 1950. At this Congress her proposal was approved for the conservation of the generic name *Candida* for the medical monilias. She was also the author of the chapter "Pathogenic Fungi" in Gay *et al.*, "Agents of Disease and Host Resistance." At the

invitation of Dr. Hilleboe, Commissioner of Health for the State of New York, she served as consultant in mycology to his department from 1948 through 1953.

Dr. Benham held membership in many scientific societies and organizations: The Society of the Sigma Xi, the Harvey Society, the Microbiological Section of the New York Academy of Medicine, the Society of Investigative Dermatology, the Society of American Bacteriologists, the New York Academy of Sciences, the American Association for the Advancement of Science, and last but not least the Mycological Society of America. She was one of the original editors of the international publication *Mycopathologia.*

In 1948 Dr. Benham's health began to fail, a heart attack required hospitalization and absence from her laboratory for nearly a year. She recovered sufficiently to resume active duty for several more years. During that time her work on the genus *Beauveria,* and additional work on the cryptococci and dermatophytes were completed. In the summer of 1955 her health failed again and she was unable to return to the laboratory. She continued writing, however, completing the manuscripts for her last two papers. After one and one-half years spent in her home in Cedarhurst, she died on January 17, 1957. Dr. Benham was an Episcopalian and was buried in her family parish in Hewlett, Long Island, New York. Her ill health had forced her premature retirement as Associate Professor in the Department of Dermatology at Columbia.

Perhaps the greatest honor a person could receive, the recognition of friends and former students, was granted to Dr. Benham. This was expressed in the form of an album of letters and photographs contributed by over seventy of her friends, students and associates, and presented to her on her sixty-second birthday. This album gave Dr. Benham great joy and the satisfaction of knowing that, though retired, she had not been forgotten.

COLLEGE OF PHYSICIANS AND SURGEONS
NEW YORK 32, N. Y.
AND
NEW YORK STATE DEPT. OF HEALTH
ALBANY 1, N. Y.

PUBLICATIONS OF RHODA W. BENHAM

1. Monilia infections of the hands and feet. New York State Jour. Med. **29**: 793–800. 1929. (With J. G. Hopkins.)
2. Asthma due to a fungus, *Alternaria.* Jour. A. M. A. **94**: 6–10. 1930. (With J. G. Hopkins and B. M. Kesten.)

3. Sensitization to saprophytic fungi in a case of eczema. Proc. Soc. Exp. Biol. Med. **27**: 342–344. 1930. (With J. G. Hopkins and B. M. Kesten.)
4. Certain monilias parasitic on man; their identification by morphology and by agglutination. Jour. Infect. Dis. **49**: 183–215. 1931.
5. *Phoma condiogena*, an excitant in asthma: some observations on the development and cultural characteristics. Bull. Torrey Bot. Club **58**: 203–214. 1931.
6. Monilias, yeasts and cryptococci; their pathogenicity, classification and identification. Am. Jour. Pub. Health **22**: 502–504. 1932.
7. Sporotrichosis in New York State. New York State Jour. Med. **32**: 595–601. 1932. (With J. G. Hopkins.)
8. Sporotrichosis: its transmission to plants and animals. Jour. Infect. Dis. **50**: 437–458. 1932. (With B. M. Kesten.)
9. Fungus infections of the skin and its appendages occurring in Puerto Rico; a clinical and mycologic study. Arch. Derm. Syph. **25**: 1046–1057. 1932. (With B. M. Kesten, B. K. Ashford, C. W. Emmons and M. C. Moss.)
10. Vegetable parasites that attack both plant and human life. Jour. New York Bot. Garden **33**: 149–153. 1932.
11. Yeast-like fungi found on the skin and in the intestines of normal subjects; a survey of 100 persons. Arch. Derm. Syph. **28**: 532–543. 1933. (With A. McH. Hopkins.)
12. The fungi of blastomycosis and coccidioidal granuloma. Arch. Derm. Syph. **30**: 385–400. 1934.
13. The terminology of the cryptococci with a note on *Cryptococcus mollis*. Mycologia **27**: 496–502. 1935.
14. Cryptococci—their identification by morphology and by serology. Jour. Infect. Dis. **57**: 255–274. 1935.
15. Pathogenic Fungi; Chapter 45 in "Agents of Disease & Host Resistance," Gay *et al.*, Ch. C. Thomas, Springfield. 1935.
16. Experimental studies with the dermatophytes. 1. Primary disease in laboratory animals. Jour. Invest. Dermat. **1**: 451–467. 1938. (With E. D. DeLamater.)
17. Experimental studies with the dermatophytes. 2. Immunity and hypersensitivity produced in laboratory animals. Jour. Invest. Dermat. **1**: 469–488. 1938. (With E. D. DeLamater.)
18. The fungi causing deep-seated infections: their diagnostic characteristics and classification. Proc. 6th Pacific Sci. Congress **5**: 863–872. 1939.
19. The cultural characteristics of *Pityrosporum ovale*—a lipophylic fungus. Jour. Invest. Dermat. **2**: 187–203. 1939.
20. Cultural characteristics of *Pityrosporum ovale*—a lipophylic fungus. Nutrient and growth requirements. Proc. Soc. Exp. Biol. Med. **46**: 176–178. 1941.
21. The nutrient and growth requirements of *Pityrosporum ovale*—a lipophylic fungus. (Abstract) Torreya **41**: 68–69. 1941.
22. Laboratory procedures in the diagnosis of fungus diseases. Proc. New York State Assoc. Pub. Health Labs. **24**: 69–82. 1944.
23. *Pityrosporum ovale*—a lipophylic fungus. Thiamin and oxaloacetic acid as growth factors. Proc. Soc. Exp. Biol. Med. **58**: 199–201. 1945.
24. Biology of *Pityrosporum ovale*. Chapter 4 in Nickerson's "Biology of Pathogenic Fungi," Ann. Cryptogam. Pytopathol. VI, Chronica Botanica Pub. Co., Waltham, Mass. 1947.

25. *Allescheria boydii,* causative agent in a case of meningitis. Jour. Invest. Dermat. **10**: 99–110. 1948. (With L. K. Georg.)
26. Effect of nutrition on growth and morphology of the dermatophytes. 1. Development of macroconidia in *Trichophyton rubrum.* Mycologia **40**: 232–240. 1948.
27. Pigment production in the differentiation of *Trichophyton mentagrophytes* and *Trichophyton rubrum.* Mycologia **41**: 291–302. 1949. (With F. C. Bocobo.)
28. Cryptococcosis and blastomycosis. Ann. New York Acad. Sci. **50**: 1299–1314. 1950.
29. The Laboratory of Medical Mycology. The Biopsy **2**: 1–2. 1950.
30. Nutritional studies of the dermatophytes with special reference to *Trichophyton megnini* Blanchard 1896 and *Trichophyton gallinae* (Megnin 1881), comb. nov. Jour. Invest. Dermat. **18**: 453–472. 1952. (With M. Silva.)
31. An unusual finding in *Epidermophyton floccosum.* Jour. Invest. Dermat. **19**: 315–317. 1952. (With P. McCormack.)
32. Nutritional studies of the dermatophytes—effect on growth and morphology, with special reference to the production of macroconidia. Trans. New York Acad. Sci. **15**: 102–106. 1953.
33. Madduromycosis of the central nervous system. Jour. Neuropath. & Exp. Neurol. **12**: 158–168. 1953. (With S. M. Aronson and A. Wolf.)
34. The genus *Beauveria,* morphological and taxonomical studies of several species and of two strains isolated from wharf-piling borers. Mycologia **45**: 727–746. 1953. (With J. L. Miranda.)
35. Chemical analysis of the capsular substance of *Cryptococcus neoformans.* Jour. Invest. Dermat. **22**: 279–283. 1954. (With J. M. Einbinder and C. T. Nelson.)
36. Nutritional studies of the dermatophytes with special reference to the red-pigment producing varieties of *Trichophyton mentagrophytes.* Jour. Invest. Dermat. **22**: 285–294. 1954. (With M. Silva.)
37. Treatment of onychomyocis due to *Trichophyton rubrum.* A. M. A. Arch. Dermat. **71**: 52–55. 1955. (With B. M. Kesten and M. Silva.)
38. The genus *Cryptococcus:* the present status and criteria for the identification of species. Trans. New York Acad. Sci. **17**: 418–429. 1955.
39. *Cryptococcus neoformans:* "an ascomycete." Proc. Soc. Exp. Biol. Med. **89**: 243–245. 1955.
40. *Trichophyton rubrum* infections: a clinical, mycologic and experimental study. Jour. Invest. Dermat. **25**: 311–328. 1955. (With M. Silva and B. M. Kesten.)
41. The genus *Cryptococcus.* Bact. Reviews **20**: 189–200. 1956.
42. Species of *Candida* most frequently isolated from man: methods and criteria for their identification. Jour. Chronic Dis. **5**: 460–472. 1957.
43. The chlamydospores of *Candida albicans:* comparison of three media for their induction. Jour. Lab. & Clin. Med. 1957. (in press—posthumously) (With J. D. Pollack.)

NOTES AND BRIEF ARTICLES

A DEVICE TO AID IN THE DEVELOPMENT OF MYCOTIC AND OTHER SKIN INFECTIONS IN LABORATORY ANIMALS

The value of employing laboratory animals for the production and study of experimental cutaneous infections or for the evaluation of topical medications for therapy or prophylaxis of such infections is evident. In carrying out such studies, the problem usually arises of how to prevent the animal from licking or biting the site of operation, thereby removing any materials applied. Bandages or similar protective devices to cover the area of operation are employed by many investigators to overcome these difficulties. However, such protective coverings are not entirely satisfactory. They take time to apply and remove, the animal often succeeds in removing the bandages, and most important, they interfere to some extent with the action of the topically applied inoculum or medication.

The purpose of this paper is to describe a simple, inexpensive method which we have found to be very satisfactory for use on laboratory animals, particularly rabbits and guinea pigs, for the prevention of licking or chewing surface inoculations or lesions. This device consists of a rubber collar of sheet rubber. An inner tube of an automobile tire is an excellent source of such material. A disc of about $4\frac{1}{2}$ inches in diameter with a central round opening of about 1 inch in diameter is satisfactory for an average sized guinea pig. The collar is simply stretched and slipped over the head of the animal. It is essential that the collar be sufficiently wide to prevent the animal from licking the site of operation. This can be determined by turning the animal's head completely to one side and making certain that the edge of the collar extends beyond the tip of the mouth. It is also necessary that the central opening be of such a size to prevent the insertion of a foot through the opening. However, it should not interefere with breathing.

The collar described can be fashioned in a few minutes. If properly made, it does not interfere with the animal's movements or with the partaking of food or water. Since the collar is light in weight, it does not cause any apparent discomfort. We have left such collars in place for a period of two weeks without interference with normal growth or weight gain.

Such protective collars have proven to be invaluable in our experimental ringworm studies in laboratory animals. In our cases, the original inoculum was still present on the animal's body at the end of two weeks. In addition, the development of lesions could be followed without the necessity of removing cumbersome bandages or tapes. The same collars have been washed, disinfected and reemployed on other animals. FIG. 1 shows a guinea pig with this protective device in place.—WILLIAM KAPLAN AND LUCILLE K. GEORG, Communicable Disease Center, Public Health Service, U. S. Department of Health, Education and Welfare, Atlanta, Georgia.

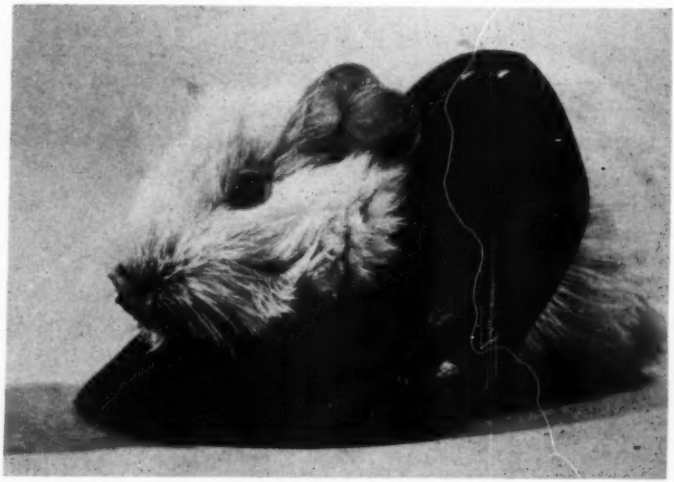


FIG. 1.

LABORATORY REFRESHER TRAINING COURSES

The Communicable Disease Center of the Public Health Service will repeat its series of refresher courses. Of particular interest to mycologists are: Laboratory methods in . . . cutaneous, subcutaneous and systemic fungi (Jan. 6–31); Laboratory methods in . . . pulmonary mycoses (Feb. 3–14); Laboratory diagnostic methods in veterinary mycology (Feb. 24–28); Serological methods in . . . parasitic and mycotic infections (Mar. 10–21).

Information and application blanks may be secured from the Laboratory Branch, Communicable Disease Center, U. S. Public Health Service, P.O. Box 185, Chamblee, Georgia.

DEATH OF DR. BESSEY

As we go to press, word has been received of the death of Dr. E. A. Bessey, at East Lansing, July 17th. Dr. Bessey was to have given the Annual Lecture at Palo Alto in September; there will be no lecture this year.

REVIEWS

BIOLOGY AND CONTROL OF THE SMUT FUNGI, by G. W. Fischer and C. S. Holden. x + 622 pp., 107 figs. Ronald Press Co., New York. 1957. Price $10.00.

The present volume is the third of a series on the subject of the smut fungi. The first was a classified subject matter guide, the second, a taxonomic treatment and this third might well be called a plant pathological treatment. Following an introductory portion on morphology, taxonomy and symptomatology, a chapter on history and economic importance, and one on nomenclature and phylogeny, the remainder of the book (340 pages) is given over to the plant pathological aspects. The whole is concluded by an excellent bibliography and an adequate index.

On the whole the treatment is thorough and the material covered well chosen. The authors have accepted the interpretations of the original workers without sufficient critical judgment in some cases. For example, in their discussion of the origin of the haplophase of the smut life-cycle they have followed the interpretation of Hüttig concerning the reduction divisions literally, thus reversing the accepted order of the homo- and hetero-typic divisions as they occur in other plants and animals. In the opinion of the reviewer Hüttig's figures do not warrant such a reversal. "Crossing-over" would explain the resultant separation of sex compatibility factors in the second division without violating the meiotic pattern of other biologic material. The authors' treatment is entirely defensible on the ground of accurate reporting and its acceptance becomes an academic point that in no way detracts from the practical results of the meiotic sequence. Their acceptance in this case partially explains their statement (p. 196) that "we are forced to admit that we cannot comprehend his (Whitehouse) interpretations" of mating-types in the case of smut heterothallism.

The competence of the authors as active investigators in this field is evident throughout this book, making it a must for the libraries of cereal pathologists and a welcome summary of our knowledge of this important and complex group of fungi.—J. C. GILMAN.

UREDINOLOGICAL STUDIES, by Naohide Hiratsuka. x + 382 pp., 79 figs., 10 pls. with 62 figs. Kasai Publishing Co., Tokyo, 1955. Price $10.00, from the author.

This volume, in Japanese, represents a treatment of the general features of the rust fungi, somewhat on the order of Arthur *et al.*, "The Plant Rusts." There are 19 chapters in which are treated the vegetative and reproductive organs, morphology, life histories, classification, host plants, heteroecism, phylogeny, distribution, specialization, hyperparasites, dissemination, etc. Chapter 16 is devoted to the rust fungi that cause diseases of economic plants in Japan. Chapter 19 consists of a list, with host plants, of all species that occur in the Japanese Archipelago. The volume is well illustrated. There are extensive literature citations with each chapter and an appended bibliography (1859–1955) of the literature dealing with Japanese rust fungi. Dr. Hiratsuka is Professor of Phytopathology and Mycology, Faculty of Agriculture, Tokyo University of Education, Ikejiri-cho, Setagaya-ku, Tokyo.—GEORGE B. CUMMINS.

TALAJBIOLÓGIA, by D. Fehér, with sections by L. Varga and O. Hank. 1263 pp. Akadémiai Kiadó, Budapest. 1954.

This volume, written in the Hungarian language, is a compendium of soil biology, starting with the microorganisms of the soil and working up through bacteria, fungi, algae, protozoans to the animals which live in the soil and the plants whose roots penetrate into the soil. Classification of microorganisms, their physiology and biochemistry are given space. Water, air, nitrogen, rhizosphere, mycorrhizae and other phases of soil biology are discussed. Various ecological factors, from the point of view of autecology, synecology of special groups of organisms and the interaction of various groups of organisms, are given prominent place in the text. It would appear to the reviewer that if the proper channels could be developed, a most useful contribution to the literature of soil biology could be made available through a translation of this book. —WM. BRIDGE COOKE.

., 79
Price

fea-
The
geta-
tion,
per-
ungi
sists
rchi-
ture
955)
a is
ture,
o.—

l O.

ium
king
live
assi-
iven
ases
oint
and
ment
oper
ture
ook.

MANUSCRIPT

Publication in MYCOLOGIA is ordinarily restricted to those who have been members in good standing of the Mycological Society of America for over a year immediately preceding submission of manuscript. Exceptions to this regulation require a favorable vote by a majority of the Editorial Board. When a paper has two or more authors, the person submitting the paper is expected to be a member.

Papers should be submitted in duplicate, typewritten and *double-spaced throughout*, to any member of the Editorial Board. When papers are not submitted in proper form, it may be necessary to return them to authors. They will be published in approximate order of their acceptance, except for the address of the retiring President and papers whose cost of publication is paid by the authors, the latter run as excess pagination.

All illustrations should be numbered consecutively throughout a paper, using arabic numbers and small letters for subdivisions, e.g., Fig. 1, a etc. This does not mean that all figures grouped for convenience on a single page need have a single number. Figures should be prepared so that, when reduced, the width will not exceed 4 inches, and should be short enough to permit the insertion of the legend beneath the figures. Each article will be restricted to twenty pages, including illustrations, except when authors submit only one paper in two or three years of membership, in which case the restriction will be thirty and forty pages respectively. Ruled tabular matter is counted double. Should an author wish to publish additional pages in one article he may do so by paying for the excess pages at current rates.

Citations of literature should be *double-spaced*, arranged in alphabetical order and cited by numbers or dates. In citing papers with two or more authors, only the first author should have the initials after the surname. The address of the author should appear at the end of the text, before the bibliography.

Each author will be restricted to two pages of half-tone illustrations or three of zinc line-engravings for each article, the total cost not to exceed $25. If figures are mounted on board, the cost of routing may be such as to restrict the space allowance substantially. Should the cost of cuts exceed $25, the author will be asked to pay the excess.

To comply with the International Rules, it is recommended that contributors furnish *brief* Latin diagnoses of all new species and genera when their manuscript is submitted for publication.

PRICE LIST OF REPRINTS

	4pp. 1 to 4	8pp. 5 to 8	12pp. 9 to 12	16pp. 13 to 16	20pp. 17 to 20	24pp. 21 to 24	28pp. 25 to 28	32pp. 29 to 32
50 Copies	$4.40	$7.00	$11.00	$11.45	$15.00	$17.15	$19.80	$21.15
100 Copies	5.25	8.35	12.75	14.10	18.00	21.15	24.65	26.45
Additional Copies per C...	1.75	2.65	4.40	5.30	6.00	8.00	9.70	10.60

For 500 copies deduct 5%; for 1000 copies or more deduct 10%.

Covers: For first 50 covers, $4.80; additional $3.45 per C.

For more than 32 pages add cost per schedule to make total. Example: for 44 pages add cost for 32 pages and 12 pages.

Note: For any reprints requiring additional composition or changes, either in text or cover, an extra charge will be made.

LANCASTER PRESS, INC.
LANCASTER, PA.

Partial List of Publications of The New York Botanical Garden

Mycologia, bimonthly; devoted to fungi, including lichens; containing technical articles and news and notes of general interest. $8.50 a year; single copies $1.75 each.

Established by The New York Botanical Garden in 1909, in continuation of the Journal of Mycology, founded by W. A. Kellerman, J. B. Ellis, and B. M. Everhart in 1885. Edited by William Alphonso Murrill, 1909–1924. Edited by Fred Jay Seaver, 1924–1946; by Alexander H. Smith, 1946–1950. Beginning with January, 1933, the official organ of the Mycological Society of America.

North American Flora. Descriptions of the wild plants of North America, including Greenland, the West Indies, and Central America. Planned to be completed in 34 volumes. Roy. 8vo. Each volume to consist of four or more parts. [Not offered in exchange.] Volumes 1–10 devoted to fungi.

Vol. 1, part 1, 1949. Myxomycetes. $7.25.

Vol. 2, part 1, 1937. Blastocladiaceae, Monoblepharidaceae, Saprolegniaceae, Ectrogellaceae, Leptomitaceae. $2.00.

Vol. 3, part 1, 1910. Nectriaceae-Fimetariaceae. $2.00. (Out of print.)

Vol. 6, part 1, 1922. Phyllostictaceae (pars). $2.00.

Vol. 7 (now complete), parts 1–15, 1906–1940. Ustilaginaceae-Aecidiaceae. $2.00 per part. (Parts 1–5 out of print.)

Vol. 9 (now complete), parts 1–7, 1907–1916. Polyporaceae-Agaricaceae (pars). $2.00 per part. (Parts 1–3 out of print.)

Vol. 10, part 1, 1914; parts 2 and 3, 1917; part 4, 1924; part 5, 1932. Agaricaceae (pars). $2.00 per part.

Series II, part 1, 1954. Tuberales. $1.60.

The New Britton and Brown Illustrated Flora of the Northeastern United States and Adjacent Canada. By Henry A. Gleason. 3 volumes. List price $30.00 per set; shipping charge $0.50. Succeeds the Illustrated Flora by Nathaniel L. Britton and Addison Brown. Includes descriptions and drawings of the plant species, from ferns to orchids, which grow without cultivation in the area extending from the St. Lawrence River to Virginia and westward to Missouri and Minnesota.

The Garden Journal of The New York Botanical Garden. Bimonthly, illustrated, containing news, book reviews, and non-technical articles on botany, exploration, and horticulture. Free to all members of the Garden. To others, 35 cents a copy, $2.00 a year. Now in its third volume. A continuation of the Journal of The New York Botanical Garden, fifty-one volumes.

Brittonia. A series of botanical papers. Subscription price, $7.50 per volume. Now in its eighth volume.

NEW YORK BOTANICAL GARDEN
Bronx Park, New York 58, N. Y.

MYCOLOGIA

OFFICIAL ORGAN OF THE MYCOLOGICAL SOCIETY OF AMERICA

Vol. XLIX SEPTEMBER-OCTOBER, 1957 No. 5

CONTENTS

[MYCOLOGIA for July–August, 1957 (49: 449–608) was issued September 6, 1957.]

PUBLISHED BIMONTHLY FOR

THE NEW YORK BOTANICAL GARDEN

AT PRINCE AND LEMON STS., LANCASTER, PA.

Entered as second-class matter April 30, 1925, at the post office at Lancaster, Pa., under the Act of August 24, 1912.

MYCOLOGIA

Published by

THE NEW YORK BOTANICAL GARDEN

IN COLLABORATION WITH THE

MYCOLOGICAL SOCIETY OF AMERICA

MYCOLOGIA

OFFICIAL ORGAN OF THE MYCOLOGICAL SOCIETY OF AMERICA

VOL. XLIX SEPTEMBER–OCTOBER No. 5

PHYSIOLOGY OF THE CELL SURFACE OF NEUROSPORA ASCOSPORES. III. DISTINCTION BETWEEN THE ADSORPTIVE AND ENTRANCE PHASES OF CATION UPTAKE[1]

R. J. LOWRY, A. S. SUSSMAN AND B. VON BÖVENTER

(WITH 7 FIGURES)

In the study of permeability changes in Neurospora ascospores, as a function of dormancy and its interruption, it was shown that the surface of the cell adsorbs several organic materials. Thus, basic dyes like methylene blue (Sussman and Lowry, **1955**) and a basic polypeptide, Polymyxin-B (Lowry and Sussman, **1956**), are localized on the surface of the dormant spore. The evidence was based on kinetic studies of uptake, elutability by other cations, and the ability of fragments of cell walls to remove such substances from solution. These data suggested that inorganic as well as organic cations are taken up by the surface of the ascospores so that this possibility was explored in the following experiments. In addition, the relation of such adsorption to subsequent penetration was undertaken in an attempt to delineate the role of surface binding in the physiology of the ascospore.

[1] This work was made possible by grants from the Rackham Graduate School, University of Michigan, and the American Cancer Society.

[MYCOLOGIA for July–August, 1957 (**49**: 449–608) was issued September 6, 1957.]

MATERIALS AND METHODS

Ascospores were grown, stored and prepared for use as described by Sussman (**1954**). Activation of the dormant cells was accomplished by heat (Goddard, **1935**) wherein the ascospores were suspended in water for 10 minutes at 58° C. Germination was studied by incubating 2 ml of a spore suspension, after the appropriate treatment, in a 25 ml Erlenmeyer flask on a reciprocal shaking machine at 30° C for 3 to 4 hours. At this time 5 drops of formaline were added in order to kill the spores whereupon counts were made, as described before (Sussman, **1954**). The LD_{50} was computed by interpolation from graphical data utilizing at least 6 points on a dosage-toxicity curve.

Thorium determinations were carried out according to the method of Hall (**1948**) wherein the optical density of the complex with carminic acid was measured at 580 mμ in a Beckman spectrophotometer. Aluminum was determined by a method identical with the above except that only 0.2 ml of the samples and standards were used while the optical density was measured at 550 mμ. By this means amounts of aluminum between 0.1 and 25 μg per ml could be measured.

Assays of uranyl ions were performed by means of the salicylate procedure recommended by Thomason *et al.* (**1955**) except that the Beckman spectrophotometer was used with 3 ml cuvettes such that amounts as low as 2 μg per ml could be measured.

Silver concentrations were studied by use of rhodanine (p-dimethylaminobenzylidene) supplied by Eastman Chemicals, as described by Sandell (**1950**). Counting of Ag^{110} was accomplished by means of an end window Geiger tube and scaler. Aluminum pans containing 0.4 ml of the solution whose Ag^{110} content was to be determined were used and enough counts were recorded to assure less than 2% counting error.

Analysis of copper was carried out by the method of Jerôme and Schmitt (**1954**) wherein the optical density of the colored complex of the metal with 2,2'biquinoline was measured at 545 mμ with the Model DU Beckman spectrophotometer. The following amounts of the reagents were used (in ml): sample, 1.0; water, 3.8; sodium acetate (sat'd), 2.4; hydroxylamine (sat'd), 0.2; ethanol, 4.0; 2.2'biquinoline, 0.2.

RESULTS

As a preliminary to the study of the surface binding of minerals, their toxicity to ascospores of Neurospora was investigated. To accomplish this 1 ml of a suspension of activated spores (1 mg per ml) in distilled water was mixed with an equal volume of enough of a mineral

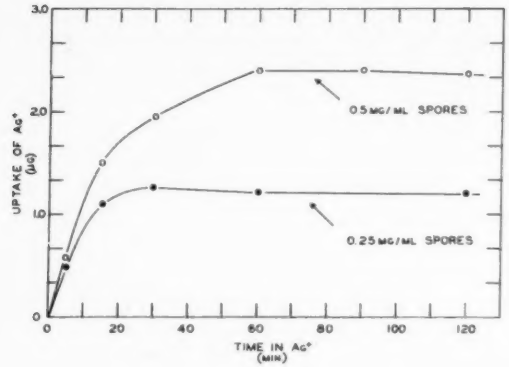
solution to give the desired final concentration. The effects of these substances were studied in concentrations ranging from 1×10^{-2} M through 1×10^{-7} M. Incubation and counting were performed as described previously, with the results shown in TABLE I. With the exception of Zn^{++}, Co^{++} and Ba^{++} the LD_{50} for these substances lies between 5×10^{-5} M and 2×10^{-6} M. The aforementioned cations are much less toxic than the latter, ranging in LD_{50} from 1×10^{-3} M to 2×10^{-4} M.

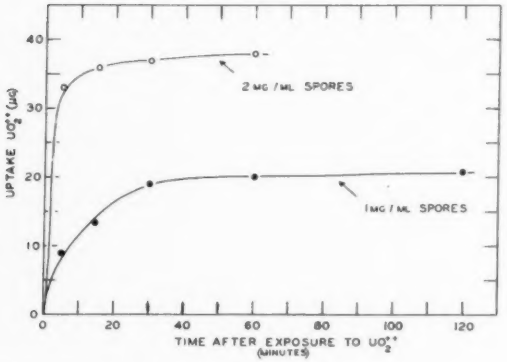
TABLE I

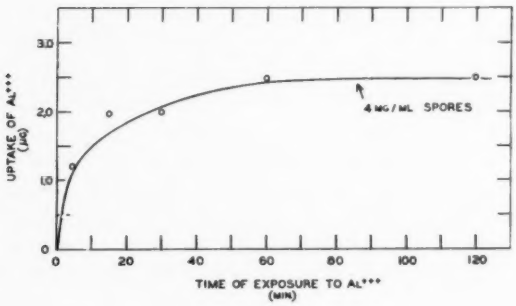
TOXICITY OF CATIONS FOR ASCOSPORES OF N. TETRASPERMA AFTER BRIEF EXPOSURE AND CONTINUAL INCUBATION. FOR BRIEF EXPOSURE, DORMANT ASCOSPORES WERE INCUBATED IN SOLUTIONS OF VARIOUS CATIONS FOR 30 MINUTES. THE SPORES WERE THEN CENTRIFUGED IN FOUR CHANGES OF DISTILLED WATER IN ORDER TO REMOVE THE RESIDUAL CATION. RESULTS ARE GIVEN AS THE AVERAGE OF 2 EXPERIMENTS. ALL METALS WERE USED AS THE CHLORIDES EXCEPT Ag^{+} AND UO_2^{++} WHICH WERE USED AS THE NITRATES.

Cation	Continuous incubation	"Coated" ascospores	
	LD_{50}	Concentration	Percentage inhibition
PCB	2×10^{-6}M	5×10^{-6}M	100
Ag^{+}	2×10^{-6}	1×10^{-4}	100
Ce^{+++}	8×10^{-6}	1×10^{-3}	67
Cd^{++}	9×10^{-6}	1×10^{-2}	100
		1×10^{-3}	65
Pb^{++}	1×10^{-5}	5×10^{-3}	36
UO_2^{++}	2×10^{-5}	1×10^{-4}	100
		5×10^{-5}	22
Au^{+++}	2×10^{-5}	1×10^{-2}	0
Th^{++++}	2×10^{-5}	1×10^{-2}	0
Cu^{++}	3×10^{-5}	1×10^{-2}	84
La^{+++}	3×10^{-5}	5×10^{-3}	35
Hg^{++}	4×10^{-5}	1×10^{-2}	13
Al^{+++}	5×10^{-5}	5×10^{-3}	0
Zn^{++}	2×10^{-4}	—	—
Co^{++}	8×10^{-4}	1×10^{-2}	0
Ba^{++}	1×10^{-3}	1×10^{-2}	0

In order to determine whether these minerals and p-chloromercuribenzoate (PCB) could be adsorbed by the cell surface, dormant ascospores were exposed to solutions of the cations used before for 30 minutes, after which they were washed and resuspended in 4 changes of distilled water. This treatment served to removed the solute from the suspending medium as evinced by negative tests for chloride. The percentage germination was then determined after activation and incubation as before. Since the slopes of the dosage-response curves for the different metals were widely disparate in these experiments, it was decided not to use LD-50 values because of the difficulty in their inter-


UPTAKE OF Ag+ (µg)
0.5 MG/ML SPORES
0.25 MG/ML SPORES
TIME IN Ag+ (MIN.)


UPTAKE UO2++ (µg)
2 MG/ML SPORES
1 MG/ML SPORES
TIME AFTER EXPOSURE TO UO2++ (MINUTES)


UPTAKE OF Al+++ (µg)
4 MG/ML SPORES
TIME OF EXPOSURE TO Al+++ (MIN.)

FIGS. 1–3.

pretation. Nevertheless, the results provided in TABLE I disclose that, as might be expected, much greater amounts of the cations are required to poison the spores as a result of brief exposure than after continual exposure during the course of incubation. In addition, metals like Hg^{++}, Pb^{++}, and La^{+++} do not completely inhibit germination even at concentrations that are 100-fold higher than those used in the previous experiment. Finally, there are metals like Th^{++++} and Al^{+++} which do not poison under these conditions even at concentrations as high as 1×10^{-3} M and 5×10^{-3} M, respectively.

These observations were furthered by experiments in which cells were exposed to several concentrations of UO_2^{++} for different intervals of time after which they were washed free of the metal, activated and incubated. Counts were then made and it is clear from these data that less than 15 minutes exposure of UO_2^{++} at 1×10^{-4} M suffices to kill almost all of the ascospores.

Quantitative analysis of the uptake of several cations was performed in order to supplement these observations. With this in mind, various concentrations of ascospores were mixed with an equal volume of 100 μg per ml of a solution of the mineral in distilled water and incubated at 20° C on a reciprocal shaking machine. Aliquots were withdrawn periodically and the spores removed by centrifugation or filtration through a fine-grade sintered glass filter. Analyses of the supernatant were performed as described previously in order to measure the residual mineral and uptake was calculated by difference. The results given in FIGS. 1–5 demonstrate that in every case the kinetics of uptake resemble those for adsorption isotherms and that the amount of uptake is a linear function of the cell concentration.

A comparison of the maximal uptake of cations by dormant ascospores and the LD_{50} is provided in TABLE II. In this table it is evident that the cations which are adsorbed in smallest amounts are those that are least toxic when tested as a "coating" as described in the protocol of the experiments whose results are summarized in TABLE I. The "adsorption ratio" provided in TABLE II appears to be an expression of the effectiveness of the cation as a toxic "coating" material.

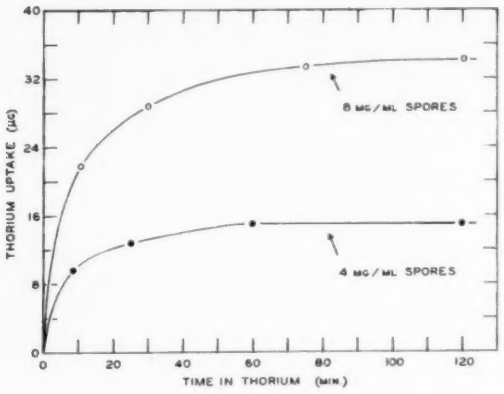
That the amount of uptake is also a function of the starting concen-

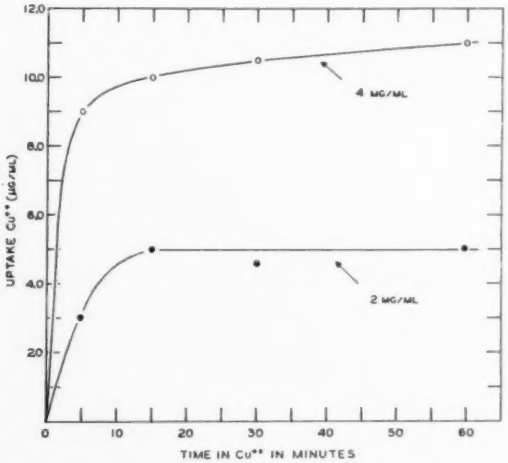
FIGS. 1–3. Uptake of cations by dormant ascospores of *N. tetrasperma*. A starting concentration of 50 μg per ml was used in all cases.

1 (top). Uptake of Ag^{+} by different concentrations of dormant ascospores.

2 (middle). Uptake of UO_2^{++} by different concentrations of dormant ascospores.

3 (bottom). Uptake of Al^{+++} by dormant ascospores.





FIGS. 4–5. 4 (above). Uptake of Th^{+++} by different concentrations of dormant ascospores of *N. tetrasperma*. A starting concentration of 50 µg per ml was used.

5 (below). Uptake of Cu^{++} by different concentrations of dormant ascospores of *N. tetrasperma*. A starting concentration of 50 µg per ml was used.

TABLE II

LD50 AND UPTAKE OF CATIONS BY DORMANT ASCOSPORES OF NEUROSPORA TETRASPERMA. CALCULATED FROM DATA IN TABLE I AND FIGS. 1–5

Cation	LD_{50} μM/mg spores	Uptake		Absorption ratio*
		μM/mg spores	Molecules/spore	
Ag^{+}	0.002	0.077	2.6×10^{11}	38.5
UO_2^{++}	0.02	0.089	3.0×10^{11}	4.4
Cu^{++}	0.03	0.043	1.4×10^{11}	1.4
Th^{++++}	0.04	0.021	1.2×10^{10}	0.5
Al^{+++}	0.05	0.030	1.0×10^{11}	0.6

* The absorption ratio is the ratio of uptake (μM per mg spores) to the LD_{50} of the mineral concerned.

tration is demonstrated in FIG. 6. In these experiments, two different spore concentrations were incubated in several concentrations of Th^{++++} at 20° C for 3 hours after which the spores were removed by centrifugation and the residual Th^{++++} determined.

Moreover, the pH also influences the amount of uptake as is shown by experiments wherein Al^{+++} and Ag^{+} solutions were made up to pH 2.0 with HCl and HNO_3, respectively. Enough ascospores were added to an equal volume of these metals to make a suspension equivalent to 4 mg per ml, after which they were incubated at 20° C on a shaking

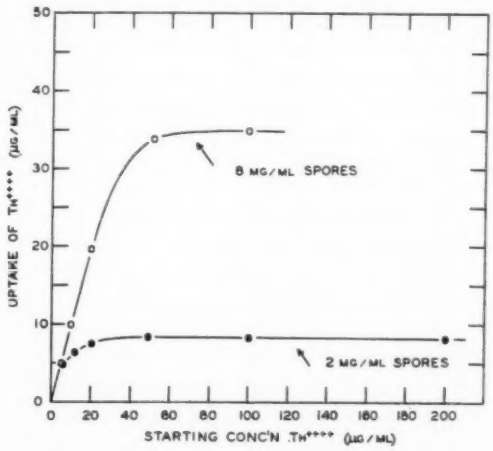


FIG. 6. Effect of varying Th^{++++} concentrations upon the uptake of Th^{++++} by dormant ascospores of *N. tetrasperma*.

machine. Analysis of samples withdrawn periodically disclosed that no Al^{+++} was taken up under these circumstances. In the case of Ag^{+} only about 7 μg per ml were absorbed as compared with the controls at pH 6.0 which removed 38.4 μg per ml from solution. It might be added that killing the ascospores by boiling in water for 15 minutes did not appreciably alter the amount of uptake of any of the five minerals used in these experiments.

On the basis of the foregoing evidence it appeared likely that the cations studied are localized on the surface of the dormant spore and do not penetrate to sensitive sites within the cell until just before protrusion of the germ tube. A likely means by which toxic cations might exert their effect is by migration from surface sites to sensitive loci within the cell. If this were so it might be expected that the affinity of the latter binding sites for the cations would have to be great enough to permit their transfer from the original sites on the surface. With this concept in mind, experiments were devised to explore the nature of the binding sites on the cell surface by comparing their affinity for certain cations with that of several compounds of physiological significance. In order to accomplish this, a suspension containing 1 mg per ml of dormant ascospores was mixed with an equal volume of 2×10^{-2} M of the salt of a toxic cation and incubated on a shaking machine at 20° C for 30 minutes. The ascospores were washed free of the residual salt by centrifugation in three changes of distilled water and resuspended to a concentration of 1 mg per ml in water. Several concentrations of various substances were then added to an equal volume of these "coated" ascospores and the mixture shaken as before for 30 minutes. The washing procedure was repeated and the ascospores were resuspended to their previous concentration in water after which they were heat-activated, incubated, killed and their germination counted as described previously.

The results of these experiments in which the competition for Ag^{+}, UO_2^{++}, PCB, Cu^{++}, and Ce^{+++} was studied are provided in TABLE III. Since cells "coated" with any of the cations tested showed greatly reduced germination when incubated in water alone, any increase in germination could be attributed to elution by the substances in which the spores were incubated. Therefore, the percentage germination was a direct measure of the affinity of these chemicals for the several cations usd as "coating" materials. It is at once apparent that the inorganic cations tried were ineffective as eluents except when spores coated with Cu^{++} were used. Of the other substances tried, the sulfhydryl-containing compounds, cysteine and thioglycollic acid, were most effective.

TABLE III

REVERSAL OF METAL TOXICITY BY VARIOUS SUBSTANCES. DORMANT ASCOSPORES OF N. TETRASPERMA WERE SHAKEN WITH AN EQUAL VOLUME OF 2×10^{-2}M OF THE METAL FOR 30 MINUTES EXCEPT FOR PBC WHICH WAS USED AT 2×10^{-3}M. AFTER BEING WASHED FREE OF THE CATION THE "COATED" ASCOSPORES WERE SHAKEN IN SOLUTIONS CONTAINING VARIOUS CONCENTRATIONS OF THE SUBSTANCES TO BE TESTED. ACTIVATION AND INCUBATION OF ASCOSPORES WAS ACCOMPLISHED AFTER THE REVERSING MATERIALS WERE REMOVED FROM THE ASCOSPORES BY CENTRIFUGATION IN 4 CHANGES OF DISTILLED WATER.

Reversing compound	Percentage germination									
	Concentration tested (M)									
	Ag^+		UO_2^{++}		PCB		Cu^{++}		Ce^{+++}	
	10^{-2}	10^{-3}	10^{-2}	10^{-3}	10^{-2}	10^{-3}	10^{-2}	10^{-3}	10^{-2}	10^{-3}
Thioglycollate	90	90	—	90	—	94	—	0	—	80
Cysteine	91	93	—	0	—	94	95	93	—	23
Dihydroxyphenylalanine (dopa)	92	90	17	4	88	0	85	75	13	21
p-aminobenzoate	0	—	70	—	0	—	26	—	88	—
Arginine	50	—	51	—	0	—	26	—	61	—
Glycine	0	—	2	—	0	—	93	—	23	—
Versene (EDTA)	0	—	72	—	91	—	97	—	92	—
Histidine	0	—	16	—	16	—	93	—	—	—
Adenosine triphosphate	97	—	—	—	–	—	92	—	—	—
Tartaric acid	21	—	—	—	0	—	59	—	85	—
Benzimidazole (5×10^{-3}M)	0	—	0	—	0	—	8	—	—	—
Na^+	0	—	0	—	0	—	6	—	—	—
K^+	0	—	0	—	0	—	16	—	—	—
Mg^{++}	0	—	0	—	0	—	41	—	—	—
Ca^{++}	0	—	0	—	0	—	82	—	—	—
Control ("coated")	0		0		0		16		0	
Control in water	91		94		93		94		92	

Here again, however, there are exceptions as in the case of Ce^{+++} and UO_2^{++} wherein cysteine was only of restricted effectiveness. The effect of 1×10^{-3} M thioglycollic acid upon spores coated with Cu^{++} was the reverse of that expected, since one-tenth that concentration completely reversed the metal's toxicity. It is possible that Cu^{++} was reduced to Cu^{+} which unpublished experiments of the authors have shown is of much higher toxicity than the oxidized form of this element. Of the other substances used, adenosine triphosphate (ATP), dihydroxyphenylalanine (dopa) and EDTA were most successful in the elution of toxic cations. In the latter instance only Ag^+ resisted incubation in this compound. Among the coating materials used, Ce^{+++} and Cu^{++} were most readily eluted while PCB appeared to be most selective in this respect.

TABLE IV

EFFECT OF VARIOUS SUBSTANCES UPON THE ELUTION OF Ag^{110} FROM ASCOSPORES OF N. TETRASPERMA. A FINAL SPORE CONCENTRATION OF 4 MG PER ML WAS USED WITH 1×10^{-2}M OF THE ELUENTS, AND INCUBATION WAS CONTINUED FOR 30 MINUTES AT 20° C

Treatment	Counts per min. in supernatant	Percentage elution
Total on spores	34.0	—
Control in water	0.7	2
ATP	26.3	76
Thioglycollic acid	12.9	38
Cysteine	11.3	33

It might be argued that the effect of the compounds used in the previous experiment could be ascribed to reasons other than removal of metal from the cell. For example, complexes formed *in situ*, or in the interior of the cell, might also be expected to render cations innocuous. To distinguish these possibilities from the one which assumes elution from the cell, 20 ml of a dormant spore suspension and an equal volume of Ag^{110} (1.2 μc per ml) were incubated at 20° C for 18 hours. The residual Ag^{110} was removed by centrifugation in 4 changes of water and the spore suspension made up to 8 mg per ml. Aliquots of the supernatant were then plated out and counted. One ml of ascospores and of solutions containing 2×10^{-2} M of several chemicals were mixed and incubated for 30 minutes after which the spores were removed by centrifugation and aliquots of the supernatant plated and counted as

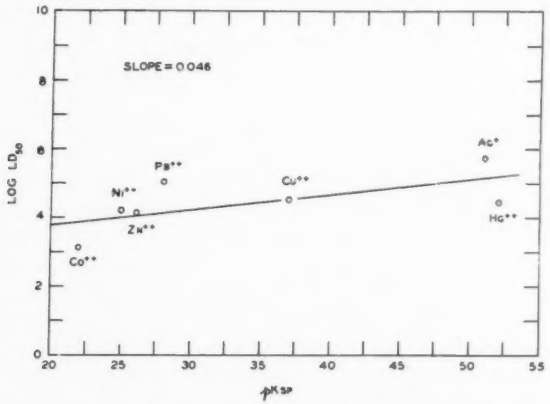


FIG. 7. Relation of the toxicity of cations to the insolubility of their mercaptides. The ordinate is the logarithm (base 10) of the LD_{50} values provided in TABLE I; the abscissa is the solubility products constant for the mercaptides of the metals whose LD_{50} is plotted as above.

described in the section on Methods. The results provided in TABLE IV disclose that a considerable portion of the Ag^{110} is eluted, as judged by the number of counts that appear in the supernatant after such treatment. It is not clear at this time why ATP is so much more effective in removing Ag^{+} than are the sulfhydryl compounds.

The effectiveness of sulfhydryl compounds in eluting cations from "coated" cells suggested the possibility that mercaptide formation in the cell was responsible for the toxic effects observed. This possibility has been discussed frequently (Albert, **1951**) and recently Shaw (**1954**) has given quantitative expression to the idea by correlating the toxicity to several organisms with the solubility product constant (pK_{sp}) for the metal sulfide. According to the equation derived during Shaw's work a plot of the negative logarithm of the metal ion concentration against the pK_{sp} will be linear. Moreover, regardless of the organism studied, the slope should be the same although the intercepts will differ. FIG. 7 illustrates the application of the Shaw plot to the data obtained in the present experiments. The slope and intercept as derived from a least squares analysis of the data are 0.046 and 2.85, respectively.

DISCUSSION AND CONCLUSIONS

These results support the notion that cations in general are adsorbed on the surface of dormant ascospores of *Neurospora tetrasperma* and do not gain access to sensitive sites in the cell until germination occurs. Support for this conclusion has been derived from the following data:

1. The kinetics of uptake resemble those for adsorption isotherms.
2. Such uptake is little affected by killing the cells.
3. Decreasing the pH of the cation solutions results in a marked reduction in uptake.
4. Uptake of cations is a function of spore concentration.
5. The starting concentration of the cation solutions greatly influences the total uptake.
6. Elution of the cations is possible by the use of chelating compounds and a variety of other substances.

In addition, previous work with this organism (Sussman and Lowry, **1955**; Lowry and Sussman, **1956**) has disclosed that absorption of cations is not markedly affected by temperature and that the effect of Polymyxin-B upon respiration does not become manifest until just before germination occurs.

The possibility of mercaptide formation being a factor in the toxicity of metals to ascospores was studied by means of the plot presented in

FIG. 7. The result was a line with a slope of 0.046 as compared with the slope of the approximately 0.062 calculated by Shaw (**1956**) from data obtained with bacteria and fungi. The significance of this discrepancy is uncertain but the difficulties inherent in experiments with these materials, including hydrolysis, differences in dissociability, etc., militate against meaningful comparisons. Furthermore, it is likely that variables such as the size of ions, charge and mobility may limit the toxicity of metals wherever barriers to penetration exist. Then, too, it seems clear that the toxicity of ions like UO_2^{++} that have little affinity for the sulfhydryl groups of proteins or amino acids (Dounce and Lan, **1949**) cannot be explained in these terms.

It has been difficult in the past to distinguish between adsorption of ions on cell surfaces and their entrance to the interior. However, the fact that cations are restricted to the exterior of dormant ascospores of *Neurospora tetrasperma* has made it possible to study the former process in the absence of the latter. As a result it has been shown that adsorption sites on the surface of the cell can serve as a reservoir of ions which penetrate as soon as germination of the cell begins. That this is so is demonstrated by the data in TABLE I wherein adsorbed inorganic ions like Ag^+, UO_2^{++}, Ce^{+++}, and Cu^{++} can kill the cell in the absence of appreciable amounts of these materials in the suspending medium. In addition, basic organic substances like Polymyxin and phenylmercuribenzoic acid can affect the cell in the same manner. It is of interest to note that similar effects have been studied by Goldsworthy and Greene (**1936**), who showed that copper is not absorbed by conidia of *Sclerotinia fruticola* until germination begins. In dealing with toxic materials like those used in the present experiments a note of caution must be introduced for it is possible that some of these can poison the cell without penetrating to its interior. For example, evidence has been presented to suggest that this is the case for UO_2^{++} (Rothstein and Larrabee, **1948**) and Polymyxin (Newton, **1954**). However, the point can still be made that access to sensitive sites is denied these materials until germination occurs, whether or not these sites are inside or on the cell surface.

On the other hand, Th^{++++} and Al^{+++} on the cell surface fail to poison the cell upon germination despite the fact that uptake does occur. In these cases, the low absorption ratio (TABLE II) may explain this observation since it is to be expected that at least as much as the amount represented by the LD_{50} (absorption ratio = 1) would have to be absorbed in order to insure some toxicity. In the case of Ag^+ and UO_2^{++}, much higher absorption ratios predict that they will be very effective poisons after adsorption has occurred. It might also be noted

that the Hofmeister series is not adhered to so far as adsorption is concerned because the tetra- and tri-valent ions are taken up in lesser amounts than are the di- and mono-valent ones.

It is tempting to speculate that the extreme toxicity of Ag^{+} for fungal cells (White, **1954**) is a function of its ability to coat the cell, thereby providing an abundance of this material for later entrance and poisoning. Although the data are too few to make generalizations possible, the work of Miller *et al.* (**1954**) showing that the bulk of Ag^{+} absorption occurred within the first few minutes of exposure suggests that the phenomenon is widespread among the fungi.

SUMMARY

The germination of ascospores of *Neurospora tetrasperma* has been shown to be inhibited by continual incubation in very small amounts of metal cations. Of these, Ag^{+}, and p-chloromercuribenzoic acid (PCB) are the most toxic. Furthermore, these cations were shown to be adsorbed to the surface of the dormant ascospore from where they can penetrate to affect sensitive loci within or upon the surface of the cell. Cations like Ag^{+}, Cu^{++} and UO_2^{++} can be taken up in sufficient quantity by the dormant cell so that germination is inhibited, even when the activated cells are incubated in distilled water. On the other hand, cations like Th^{++++} and Al^{+++} do not inhibit under the latter conditions despite the fact that they are bound to the cell surface and are quite toxic when continually incubated with the germinating ascospore. These experiments support the idea that the dormant cell is impermeable to cations whereas this barrier is raised when germination is induced. Although, in a general way, the toxicity of some of the metals tested varies directly as a function of the insolubility of their mercaptide, the slope of the curve does not agree with that found by Shaw for other organisms.

DEPARTMENT OF BOTANY
UNIVERSITY OF MICHIGAN
ANN ARBOR, MICHIGAN

LITERATURE CITED

Albert, A. 1951. Selective toxicity with special reference to chemotherapy. Methuen and Co., London.

Dounce, A. L. and T. H. Lan. 1949. The action of uranium on enzymes and proteins. In Voegtlin and Hodge, Pharmacology and toxicology of uranium compounds. McGraw-Hill and Co., New York.

Goddard, D. R. 1935. The reversible heat activation inducing germination and increased respiration in the ascospores of *Neurospora tetrasperma*. Jour. Gen. Physiol. **19**: 45–60.

Goldsworthy, M. C. and E. L. Greene. 1936. Availability of the copper of Bordeaux mixture residues and its absorption by the conidia of *Sclerotinia fruticola*. Jour. Agric. Res. **52**: 517–533.

Hall, R. H. 1948. A rapid colorimetric method for the determination of microquantities of Thorium, AECD-2437, Oak Ridge National Lab., Oak Ridge, Tenn.

Jerôme, H. and H. Schmitt. 1954. Micromethode spectrophotométrique de dosage du cuivre total dans les tissues et liquides biologiques. Bull. Soc. Chim. Biol. **36**: 1343–1354.

Lowry, R. J. and A. S. Sussman. 1956. Physiology of the cell surface of *Neurospora* ascospores II. Interference with dye adsorption by Polymyxin. Arch. Biochem. Biophys. **62**: 113–124.

Miller, L. P., S. E. A. McCallan and R. M. Weed. 1954. The use of radioisotopes for studying the affinity of various toxicants for fungus spores. In Second Radioisotope Conference, Oxford, England. Preprints 1, AU 43. Butterworths Scient. Publications, London.

Newton, B. A. 1954. The use of a compound forming fluorescent conjugates with protein in studying the mode of action of Polymyxin. Biochem. Jour. **56**: xxxii.

Rothstein, A. and C. Larrabee. 1948. The relationship of the cell surface to metabolism II. The cell surface of yeast as the site of inhibition of glucose metabolism by uranium. Jour. Cell. Comp. Physiol. **32**: 247–260.

Sandell, E. B. 1950. Colorimetric determination of traces of metals. 2d edition. Interscience Publishers, Inc., New York.

Shaw, W. H. R. 1954. Toxicity of cations toward living systems. Science **120**: 361–363.

Sussman, A. S. 1954. Changes in the permeability of ascospores of *Neurospora tetrasperma* during germination. Jour. Gen. Physiol. **38**: 59–77.

—— **and R. J. Lowry.** 1955. Physiology of the cell surface of *Neurospora* ascospores I. Cation binding properties of the cell surface. Jour. Bacteriol. **70**: 675–685.

Thomason, P. F., L. A. Nutting, U. Koskela and W. M. Byerly. 1955. The salicylate method for determining microgram amounts of uranium. ORNL 1641, Oak Ridge National Lab., Oak Ridge, Tenn.

White, J. 1954. Yeast technology. John Wiley and Sons, Inc., New York.

ACIDIC METABOLIC PRODUCTS OF POLYPORUS SULPHUREUS[1]

WILLIAM R. BUSHNELL

(WITH 4 FIGURES)

In the course of studies of metabolism of the higher fungi in this laboratory, Allen (**1952**) found that the culture medium in which an isolate of *Polyporus sulphureus* Fries[2] was growing became quite acid during extended incubation. In 250 ml of medium of initial pH 6.2 to 6.5, acidic compounds were formed in sufficient quantity to cause the pH to drop as low as 2.1 and increase the titratable acidity by approximately 3 ml of normal acid. None of 50 other species and strains of higher fungi screened by Gray and Bushnell (**1955**) formed acidic products to that extent and only a few were found which formed nearly comparable amounts of acid. For example, only six caused culture media to become more acid than pH 3.0 and only nine synthesized acidic products equivalent to more than 1 ml of normal acid per 250 ml culture. Varying degrees of acidity in the culture media of isolates of *P. sulphureus* have been reported by Curtin (**1927**), Jennison *et al.* (**1955**), and Nesemann (**1953**). Rabanus (**1939**) found that oxalic acid was produced by this fungus in quantities sufficient to reduce the effectiveness of copper sulphate treatments of structural timbers in calcareous soils. Since *P. sulphureus* synthesized more acidic metabolites than any of the other higher fungi under investigation in this laboratory, a study of acid synthesis by this organism was undertaken. Included in this report are quantitative data on acid production collected by Gray and Liu (unpublished data) and isolation and tentative identification of the principal acidic products of *P. sulphureus*.

MATERIALS AND METHODS

The organism used in these studies was isolated by means of tissue culture from the stipe of a fresh sporophore of *P. sulphureus* by Dr.

[1] Paper No. 593 from the Department of Botany and Plant Pathology, The Ohio State University.

[2] Culture No. 99 in the mycology stock culture collection of the Department of Botany and Plant Pathology, The Ohio State University.

Stuart L. Hughes in September, 1949. This organism grows only on the surface of liquid medium and develops numerous non-wettable spore-like bodies (oidia?) in the upper layers of the mycelial mat. It has failed to grow appreciably in vigorously aerated submerged culture tanks. This lack of growth is attributed at least in part to the non-wettable character of some of the components of the mycelial mats used for inoculum in these trials.

TABLE I

CONSTITUENTS OF MEDIA

CHEMICALLY DEFINED MEDIUM	
Glucose	30 gm
Potassium dihydrogen phosphate	0.2 gm
Dipotassium hydrogen phosphate·$3H_2O$	0.8 gm
Ammonium nitrate	2.0 gm
Potassium nitrate	1.0 gm
Magnesium sulfate·$7H_2O$	0.5 gm
Potassium chloride	0.5 gm
Ferrous sulfate·$5H_2O$	0.04 mg
Zinc sulfate·$7H_2O$	0.025 mg
Manganous sulfate·$4H_2O$	0.025 mg
Sodium borate·$10H_2O$	0.025 mg
Ammonium molybdate·$2H_2O$	0.025 mg
Copper sulfate·$5H_2O$	0.025 mg
Folic acid	1.3 μg
Thiamine	99.9 μg
Pyridoxin	50.2 μg
Calcium pantothenate	200.0 μg
Niacin	200.1 μg
Choline chloride	200.4 μg
p-amino benzoic acid	50.3 μg
Inositol	400.0 μg
Riboflavin	50.0 μg
Distilled water	to 1000 ml
YEAST EXTRACT MEDIUM	
Dextrose	30 gm
Difco yeast extract	7 gm
Potassium dihydrogen phosphate	5 gm
Distilled water	to 1000 ml

Two types of liquid media were used: a chemically defined medium and a yeast extract—KH_2PO_4—dextrose medium. The constituents of these media are listed in TABLE I. The chemically defined medium was developed by Allen (**1952**) and used by Gray and Bushnell (**1955**) but its constituents have not been published previously.

Rate of acid production was determined by measuring total acidity, volatile acidity, and pH of culture medium at intervals during extended incubation of cultures of *P. sulphureus*. Several series of 40 to 70 liquid cultures were prepared, each culture consisting of a 250 ml Erlenmeyer flask containing 100 ml of medium and a small piece (*ca.* 6× 6

× 2 mm) of mycelial inoculum from an agar slant. These were incubated at 25° C, single flasks being selected at intervals for analysis. The medium, including mycelium, was diluted to the initial volume of 100 ml before measurements were made. The pH was then determined with a Beckman Model H line-operated pH meter. Total acidity was determined by diluting a 5 ml sample of the medium to 100 ml with distilled water and then titrating this diluted sample with 0.01 N NaOH using phenolphthalein as indicator. Volatile acidity was determined by titration of the steam distillate of a 5 ml sample of the medium with 0.01 N NaOH, approximately 100 ml being distilled from the sample using a modified Hortvet apparatus. Non-volatile acid was then computed as the total acidity less the volatile acidity.

For purposes of collecting material for isolation and identification of the acidic products, eight to fifteen cultures were prepared, each consisting of a 500 ml Erlenmeyer flask containing 100 or 200 ml of medium. When pH and total acidity measurements of samples from a series of cultures indicated the presence of considerable acid (pH 2.0 to 2.5), the fermentation medium from these cultures was pooled, neutralized with 30 per cent NaOH, and concentrated to a syrup on a steam bath. This concentrate was acidified with 50% phosphoric acid and ether-extracted from one to ten days depending upon the amount of ether-soluble acid present. When it was desired to retain the volatile acid portion of the extract, the ether was evaporated from the extract only after neutralization with normal NaOH. When the non-volatile acids were desired in the free acid state, the ether-extracts were not neutralized and, in these cases, most of the volatile acid was lost during evaporation of the ether.

Separation and tentative identification of the acid in the extract was attempted using a silicic acid adsorption column in the manner described by Marvel and Rands (**1950**) and used in this laboratory by Hughes (**1952**). In one experiment, steam distillation was used to separate the volatile acid fraction from the ether-extracted material. A partition coefficient of the volatile acid was determined following a modification of the procedure described by Osburn *et al.* (**1936**). Fifty-five ml portions of acid solution were extracted for 1.5 minutes with 25 ml ethyl ether; 50 ml portions of both the extracted and the original solutions were then titrated.

All melting points were measured with one thermometer and are uncorrected.

RESULTS AND DISCUSSION

Rate of Acid Production.—Typical examples of the data on the rate of acid synthesis by *P. sulphureus* collected by Gray and Liu are presented in FIGS. 1 and 2. The values for maximum increase of total acidity in the yeast extract medium series at 50 days of incubation is approximately three times that in the chemically defined medium at 112 days. The decrease in total acidity in the chemically defined medium series after 112 days suggests that the acidic metabolites were being utilized by the organism at a rate greater than their synthesis in the latter part of the incubation period. In both media the amount of

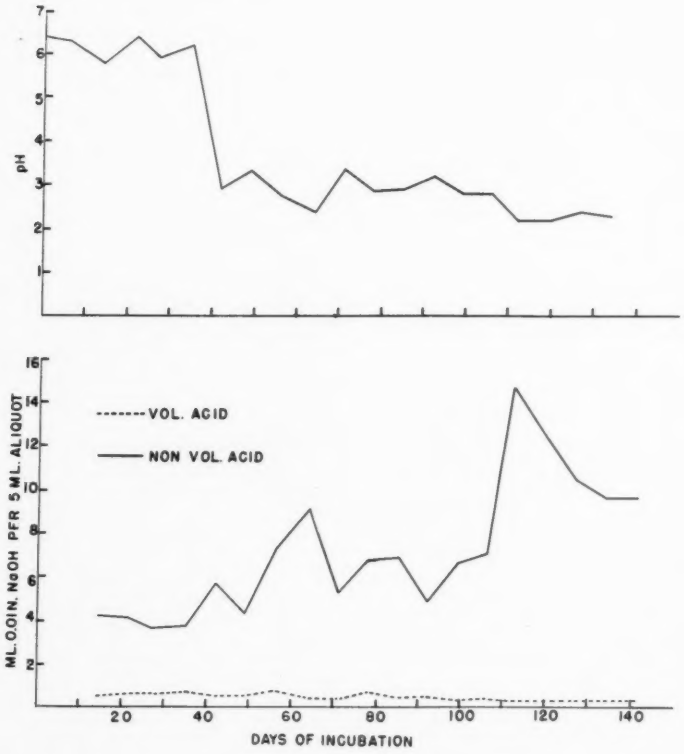


FIG. 1. Acid synthesis by *P. sulphureus* on chemically defined medium. Data of Gray and Liu.

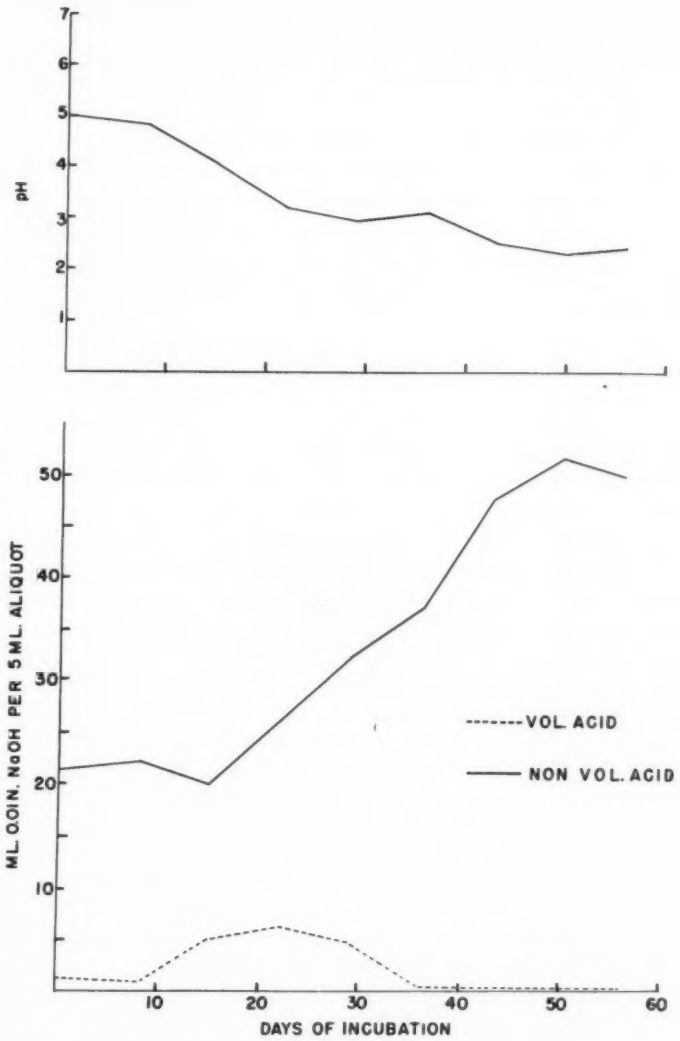


FIG. 2. Acid synthesis by *P. sulphureus* on yeast extract medium. Data of Gray and Liu.

volatile acid present in the second half of the incubation period is less than in the first half.

Acidic Components of the Medium.—Media from three different sets of cultures were used in the attempts at separation and identification of the acidic metabolites occurring in the medium in which *P. sulphureus* had been cultured. The pertinent data for each set are listed in TABLE II and references will be made subsequently to these as Groups A, B, and C as presented in the table.

TABLE II

CONSTITUENTS, LENGTH OF INCUBATION, INITIAL AND FINAL ACIDITIES OF CULTURES USED FOR ISOLATION AND IDENTIFICATION OF ACIDS SYNTHESIZED BY *P. sulphureus*

Group	Type of medium	No. of flasks	Days of incubation	Size flask (ml)	Medium per flask (ml)	Total acidity 0.01 N NaOH/ 5 ml aliquot		pH	
						Initial	Final	Initial	Final
A	Synthetic	8	132	500	200	3.5	7.0	6.0	2.5
B	Yeast Extract	8	132	500	200	20.8	77.0	5.5	2.0
C	Yeast Extract	11	41	500	100	21.2	69.0	5.4	2.3

Volatile Acid.—Evidence suggesting the presence of acetic acid in the fermentation medium of *P. sulphureus* was obtained from Group A. Following the standard procedure, this medium was concentrated and ether-extracted. A strong odor similar to that of acetic acid was noticed when the ether was nearly evaporated from the first day's extract. In order to preserve the volatile acid in the second day's extract, the ether solution was placed over distilled water and the two solvent layers neutralized with normal NaOH before the ether was allowed to evaporate. Evaporation of the ether then yielded an aqueous solution of the sodium salts of the acidic components of the second day's extract. This was placed in the Sellier tube of the modified Hortvet apparatus, acidified with phosphoric acid, and steam distilled until 70 ml of distillate was recovered. This distillate was diluted to 110 ml with distilled water and its partition coefficient determined to be 81.6. The partition coefficient of acetic acid by determination with the same glassware was 82.7. It was tentatively concluded that acetic acid or its salts were present in the fermentation medium of *P. sulphureus.*

Non-Volatile Acid.—The data of Gray and Liu, FIGS. 1, 2, had indicated that the greater portion of the acid in the culture media was non-volatile. The large yields of acidic material which were obtained from the medium of Group B (TABLE II) enabled identification of the principal acid by the physical characteristics of the acid itself.

When the medium from Group B was concentrated, in this case without prior neutralization, and left in the evaporating dish for several days, a crust of crystals formed over the syrupy concentrate as well as crude crystalline pellets one to two millimeters in diameter in the syrup on the bottom of the dish. Both types of crystals were removed from the syrup and rinsed lightly with water to remove gross impurities. The pellets were dried and found to be an acidic material weighing 1.1 gm and having a neutralization equivalent of 84.6. The other crystals, which formed as a crust on the surface, were placed in about 15 ml water and then ether-extracted for two days without acidification. The small amounts of material thus extracted had a neutralization equivalent of 64.5. Syrupy phosphoric acid was added to the crystals in the extractor and the extraction continued for ten days, at the end of which time no crystals remained in the extractor. The total weight of the extracted material was 7 gm.

Translucent crystals were deposited on the sides of the ether container during the extraction period. These crystals were removed, dried in a desiccator for 24 hours and then found to have a melting point of 98–100° C and a neutral equivalent of 67.1. These values suggested dihydrate oxalic acid for which McElvain (**1945**) lists a melting point of 101° C and a neutral equivalent of 63. With the equipment in our laboratory, the melting point of known oxalic acid [$(CO_2H)_2 \cdot H_2O$] was determined to be 98–100° C and its neutral equivalent 63.4. The unknown crystals were mixed intimately with the known oxalic acid and an undepressed melting point of 98–100° C was obtained. Oxalic acid dihydrate characteristically sublimes above 100° C forming anhydrous oxalic acid with a melting point of 186–187° C (Lange, **1944**). The unknown material when heated overnight sublimed at 100° C as did known oxalic acid—the unknown then having a melting point of 192.5–193° C and the known oxalic having a melting point of 191–194° C. The precipitate from the concentrated medium was therefore oxalic acid. The 7 gm of acid obtained represents over half the increase in titratable acidity of the medium when computed as oxalic acid dihydrate (56.2 ml 0.01 N NaOH per 5 ml aliquot).

Silicic Acid Adsorption Column.—Elution of similar material in the silicic acid adsorption column substantiated the presence of oxalic acid in the culture medium. Initially the peak effluent volumes of several known acids were determined. These known acids were placed on the column in the manner suggested by Marvel and Rands. Butanol solubles were dissolved in 0.4 ml butanol, mixed with 0.6 ml chloroform, and placed on the column. Chloroform solubles were dissolved in 1 ml

chloroform and then placed on the column. Some peak effluent volumes which resulted are indicated in FIG. 3. These peaks were usually reproduceable within ±10 ml of eluent, larger disparities occurring only with those acids eluted late in the development of the column as in the case of citric acid.

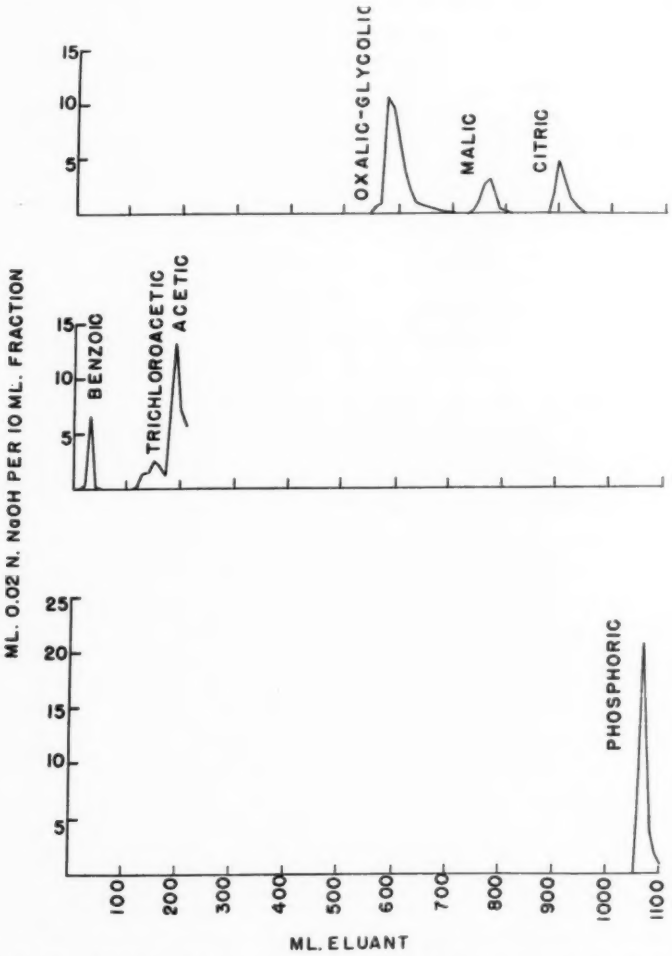


FIG. 3. Fractionation of known acids on silicic acid adsorption column. Each of the three graphs represents the elution of a single column.

Oxalic and phosphoric acids had peak effluent volumes of 570 and 1070 respectively. Comparable values were obtained by Phares *et al.* (**1952**) using somewhat different eluent proportions. Marvel and Rands list oxalic acid at 1000? and do not list phosphoric acid. They list glycolic at 577 and it was found that only one peak could be detected in this region when glycolic acid and oxalic acid were eluted on the same column.

When the medium from Group C (TABLE II) was neutralized and concentrated by the standard procedure, a precipitate formed over the syrupy concentrate similar to that formed from the medium of Group B. These crystals were removed from the syrup, rinsed lightly with dilute NaOH, acidified with phosphoric acid, and then ether-extracted for two days. This extract was neutralized and ether-extracted again to remove neutral ether solubles. The material remaining was acidified again and ether-extracted for one day. When the ether was evaporated from this extract, a soggy white residue remained which became no dryer during several days in a desiccator. Part of this residue (178.5 mg) was placed on the silicic acid adsorption column following the standard procedure.

The upper graph of FIG. 4 illustrates the titrations of the eluent from this column. The principal peak (575) occurred where trials with known acid had indicated the peak effluent volume of oxalic acid to be.

Apparently phosphoric acid was ether-extracted in small quantities in the final extraction above, since the peak at 1070 is at the same effluent volume as that obtained with phosphoric acid (bottom graph of FIG. 3). Liquid residues were found on several occasions in the ether-extracted residues and in one trial with such a liquid, a peak effluent volume of 1070 was obtained. This eluted material gave a positive test for phosphate with ammonium molybdate and stannous chloride. However, another acid with a similar peak of effluent volume such as glyceric at 1070 or tartaric at 1015 might have been present but obscured by the presence of phosphoric acid.

Several other acids were present in the syrupy concentrate of Group C. This syrup was acidified and ether-extracted for one day. In order to preserve the volatile acid fraction and also place this acidic material on the silicic acid adsorption column with a minimum of water, the material was neutralized with 9.1 ml normal NaOH and evaporated to dryness on a steam bath. Two ml of chloroform was added to the residue and 0.3 ml of concentrated sulfuric acid slowly stirred into the slurry. The chloroform solution was decanted and 2 ml of butanol added to the slurry and the stirring continued. The butanol solution

was decanted and added to 2 ml of chloroform and the chloroform-butanol mixture added to the column. Finally, the chloroform solution was placed on the column and elution started.

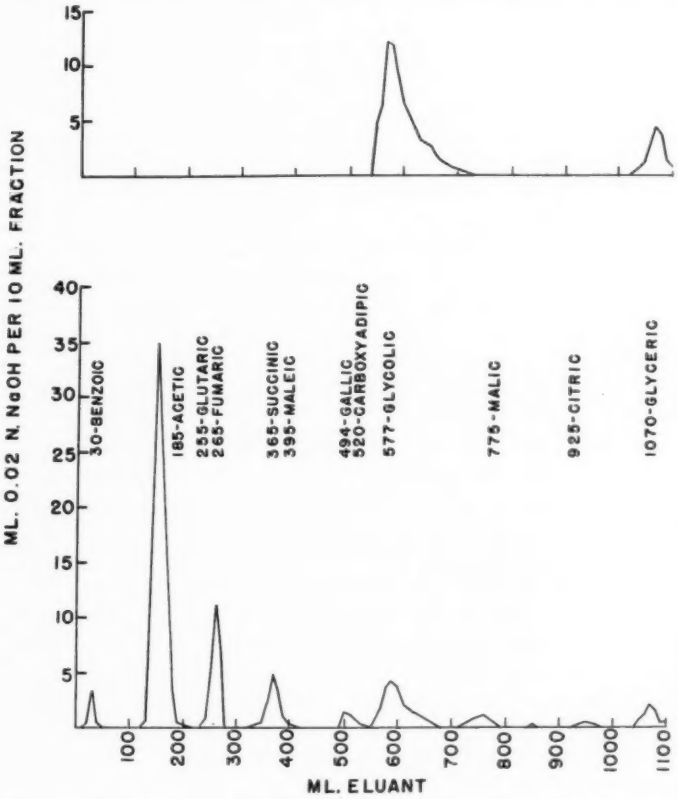


FIG. 4. Fractionation of unknown acidic material on silicic acid adsorption column. Upper graph: material from precipitate from concentrated medium of Group C. Lower graph: material from syrupy concentrate of Group C.

The titrations of the eluent from this column are shown in FIG. 4 (lower graph). The peak effluent volumes listed across the top of this graph are those listed by Marvel and Rands in the regions where peaks were detected from this unknown. In view of the evidence above for the presence of acetic acid, the fraction of eluent with a peak effluent

volume at 150 probably contained acetic acid which was eluted 35 ml early because of the large amount of butanol used to place the material on the column. The fraction with a peak effluent volume at 590 probably contained oxalic acid, small amounts undoubtedly being present in solution in the syrup from which these acids were extracted. Again the fraction which peaked at 1070 probably contained reagent phosphoric acid.

As illustrated in FIG. 4, seven other fractions of eluent contained acidic material. The peak effluent volumes of these fractions occurred at 30, 260, 370, 500, 760, 850, and 950. Since fumaric, succinic, malic, and citric acids are postulated precursors in the synthesis of oxalic acid (Foster, **1951**), the fractions with peak effluent volumes at 260, 370, 760, and 950 may have been due to these acids. The acids which Marvel and Rands indicate are eluted with peak effluent volumes at 30, 500, and 850 do not include any of the other suggested intermediates in the formation of oxalic acid from carbohydrate.

The quantitative relationships of the acids in the medium were not necessarily indicated by the magnitude of the total titrations of the acidic fractions of eluent in the graphs of FIG. 4. The ether-extractions were not prolonged to the extent that all the acid was extracted and it is not likely that the extracted material contained the acids in the same proportion as those in the original medium. The amount of oxalic acid placed on the column (upper graph) represented only a small fraction of the total oxalate which precipitated from the neutralized and concentrated medium.

General Considerations.—Although production of large amounts of free acid is probably not characteristic of the higher fungi in general (Gray and Bushnell, **1955**), free oxalic acid accumulation by members of the brown wood-rot group of fungi is particularly common. The greatest acidity of the medium of the isolate used in this study was pH 2.0. Shimazono (**1952**) reported three brown wood-rot fungi which lowered the pH of a glucose-peptone medium to 1.2 after 40 days incubation. An isolate of *Poria vaporaria* synthesized 1.7 grams acid per 100 ml medium. Jennison (**1955**) found six out of 29 brown wood-rot fungi which caused the pH of a malt extract medium to drop to pH 2.0 or below. *P. sulphureus,* also a brown wood-rot fungus, is not outstanding as an acid producer when compared with other members of this group.

Production of oxalic acid is probably characteristic of most strains of *P. sulphureus.* Also, oxalate formation by this organism is reported to occur in its native environment. Neumann (**1914**) quotes Massee as

describing its fruiting body as follows: "Specimens when dry are often more or less encrusted with a deposit of binoxalate of potash." Wilson (**1948**) found the exudate from a sporophore of *P. sulphureus* in its native environment to have pH 3.57 to 3.8.

Foster (**1949**) indicates that acetic acid is not usually found in the culture medium of fungi which are producing oxalic acid from carbohydrate, although acetic acid has been postulated as an intermediate in the process. The presence of this acid in the culture medium of *P. sulphureus* supports this hypothesis.

The existence of seven acids other than acetic and oxalic in amounts detectable in the culture medium of *P. sulphureus* by the use of the silicic acid adsorption column may be related to the slow enzymatic processes of this organism.

SUMMARY

1. Rate of acid synthesis by *P. sulphureus* on liquid media was investigated. On a yeast extract medium an increase of non-volatile acidity equivalent to 6 ml of normal acid per 100 ml of medium occurred during 50 days of incubation while the maximum volatile acidity which occurred on the twenty-third day was equivalent to 1.2 ml of normal acid and dropped to near zero values after the thirty-sixth day. On a chemically defined medium, maximum non-volatile acidity equivalent to 2 ml normal acid was obtained on the one hundred and twelfth day.

2. Identification of the acidic components of the ether-soluble material from one to two liters of medium was attempted. A partition coefficient of the steam distillate of such material indicated that acetic acid was the principal volatile acid present. Crystalline precipitates from the concentrated culture medium were found to be principally oxalic acid.

3. Fractionation of the acidic material on a silicic acid adsorption column substantiated the presence of oxalic and acetic acids and indicated that seven other acids were present in the medium. The peak effluent volumes suggested that four of these were malic, fumaric, succinic, and citric acid.

The writer is indebted to Dr. W. D. Gray for his helpful advice throughout the course of this work.

DEPARTMENT OF BOTANY
UNIVERSITY OF WISCONSIN
MADISON, WISCONSIN

LITERATURE CITED

Allen, J. P. 1952. Carbon train technique and its application to quantitative studies of Basidiomycete metabolism. Unpublished Ph.D. Dissertation, Ohio State Univ.

Curtin, L. P. 1927. Experiments in wood preservation: I. Production of acid by wood-rotting fungi. Indus. and Engin. Chem. **19**: 878–881.

Foster, J. W. 1949. Chemical Activities of Fungi. New York: Academic Press, Inc. Pp. 326–349.

——. 1951. Metabolism of fungi. Ann. Rev. Microbiol. **5**: 101–120.

Gray, W. D. and W. R. Bushnell. 1955. Biosynthetic capacities of the higher fungi. Mycologia **47**: 646–663.

Hughes, S. B. 1952. Acidic metabolic products of *Hansenula anomala*. Mycologia **44**: 736–746.

Jennison, M. W., M. D. Newcomb and R. Henderson. 1955. Physiology of the wood-rotting Basidiomycetes. I. Growth and nutrition in submerged culture in synthetic media. Mycologia **47**: 275–304.

Marvel, C. S. and R. D. Rands, Jr. 1950. Separation of organic acids. Jour. Amer. Chem. Soc. **72**: 2642–2646.

McElvain, S. M. 1945. The Characterization of Organic Compounds. New York: Macmillan Co. Pp. 185–191.

Nesemann, G. 1953. Über die antagonistische Beeinflussung von Wachstum und Atmung bei einigen höheren Pilzen. Arch. Mikrobiol. **19**: 319–352. Abs. Rev. Appl. Mycol. **34**: 418. 1955.

Neumann, J. J. 1914. The Polyporaceae of Wisconsin. Wis. Geol. and Nat. Hist. Surv. Bull. No. 33, Scientific Series No. 40: 104.

Osburn, O. L., H. G. Wood and C. H. Werkman. 1936. Determination of volatile fatty acids by the partition method. Ind. Eng. Chem., Anal. Ed. **8**: 270–275.

Phares, E. F., E. H. Mosbach, F. W. Denison, Jr. and S. F. Carson. 1952. Separation of biosynthetic organic acids by partition chromatography. Anal. Chem. **24**: 660–662.

Rabanus, A. 1939. Über die Säure—Produktion von Pilzen und deren Einfluss auf die Wirkung von Holzschutzmitteln. Mitt. dtsch. Forstver. **23**: 77–89. Abs. Rev. Appl. Mycol. **38**: 426. 1939.

Shimazono, H. and K. Takubo. 1952. The biochemistry of wood decaying fungi: I. The Bavendamm's reaction and the accumulation of oxalic acid by wood decaying fungi. Bull. For. Exp. Sta. Tokyo **53**: 117–126.

Wilson, J. K. 1948. An examination of the exudate and juice of certain fungi found in their native environment. Mycologia **40**: 605–613.

THE INACTIVATION OF PYRIDINETHIONE, AN ANTIFUNGAL AGENT, BY GLUCOSE [1]

SAMUEL M. RINGEL [2] AND E. S. BENEKE

(WITH 2 FIGURES)

Pyridine-3-sulfonic acid, 2-sulfanilamido-5-nitropyridine and isonicotinic acid hydrazide (isoniazid) are but a few of the many known pyridine containing compounds which possess antimicrobial activity. A new compound, 1-hydroxy-2(1H)-pyridinethione, has been of particular interest to us, since it exhibits excellent *in vitro* control against many fungi as well as against gram-positive bacteria (**8, 14**). Initial results against phytopathogenic fungi *in vivo* also appear promising (**2, 12**). A preliminary investigation, *in vitro* (**11**), indicated that the sodium salt of 1-hydroxy-2(1H)-pyridinethione, when tested against *Colletotrichum phomoides*, was somewhat inactivated by reducing sugars.

In this report, a further evaluation is made of the effects of glucose on the antifungal activity of pyridinethione [3] with respect to concentration and time. Spectrophotometric as well as bioassay methods were employed.

MATERIALS AND METHODS

The organism used in this study was *Colletotrichum phomoides* (Sacc.) Chester, obtained from Indiana tomato fruits in the summer of 1953 and designated as isolate C80A. Potato dextrose agar slant cultures were routinely maintained. Spore inoculum was obtained from shake cultures and used in the bioassay studies at a concentration of 3000 spores per ml of medium. The spore harvest procedure is described elsewhere (**11**).

[1] Journal article No. 2060 from the Michigan Agricultural Experiment Station and Contribution No. 17-7 from the Department of Botany and Plant Pathology, Michigan State University, East Lansing, Michigan. This work was made possible by a grant from the Horace H. Rackham Research Endowment.

[2] Present address: U. S. Market Pathology Laboratory, 641 Washington St., Rm. 1022, New York 14, N. Y.

[3] The sodium salt of 1-hydroxy-2(1H)-pyridinethione lot by Py-354-75C was kindly furnished by the Squibb Institute for Medical Research, New Brunswick, New Jersey.

A basal synthetic medium was employed following Lilly and Barnett's formulation (**7**), which, compounded on the liter basis, contained L-asparagine, 2 g; $MgSO_4 \cdot 7H_2O$, 0.5 g; KH_2PO_4, 1 g; Fe, 0.2 mg; Zn, 0.2 mg; Mn, 0.1 mg; thiamine HCl, 100 μg, and biotin, 5 μg. All chemicals used were of reagent grade purity. The complete medium, except in the sugar concentration study, contained either D-glucose, 10 g, or sucrose, 9.5 g. The medium was made up to 960 ml volume with double distilled water (so that the addition of one ml inoculum and one ml Seitz filtered sodium pyridinethione to 48 ml of the autoclaved medium would yield 50 ml at the proper concentration level of nutrients). The pH was adjusted to 6.0 with the aid of a Beckman glass electrode pH meter. Forty-eight ml of the medium were dispensed into 250 ml Erlenmeyer flasks, plugged with cotton and autoclaved at 120° C for 15 minutes. All glassware employed had been previously cleansed in a synthetic detergent solution followed by a tap water wash and a distilled water rinse. In order to prevent dehydration, screw cap bottles were used in the extended contact time series.

The inoculated culture vessels were incubated in a stationary position at 26° C for nine days. At harvest time, the mycelium from each vessel was filtered with suction on a Buchner funnel through a nylon filter. The mycelium was then washed three times with distilled water, placed in a tared aluminum weighing cup and dried overnight at 60° C. The cups containing the dried mycelia were kept in a desiccator until weighed. Each value reported is the average of three flasks. In one series where acetaldehyde was added to the sucros medium with the inhibitor, the results were assessed on the basis of visual observation of growth.

If a reaction occurred between D-glucose and sodium pyridinethione, it was considered possible to detect this spectrophotometrically by a comparison of the ultraviolet absorption spectra of the reactants and their mixture. Sodium pyridinethione, at a concentration of 4 μg/ml in distilled water, was Seitz filtered to duplicate the routine procedure (the lower concentrations used in the bioassay evaluations could not readily be detected spectrophotometrically). Separate solutions of D-glucose and sucrose were prepared in distilled water and autoclaved for 15 minutes at 120° C. Mixtures were prepared by combining equal volumes of the sugar and the pyridinethione solutions, each at double strength. The ultraviolet absorption spectra were determined with the aid of a Beckman Quartz Spectrophotometer, Model DU, employing matched quartz cuvettes.

THE EFFECT OF ACETALDEHYDE UPON SODIUM PYRIDINETHIONE

In order to determine whether inactivation of sodium pyridinethione is due to the presence of free carbonyl or aldehyde groups found in reducing sugars, acetaldehyde was added to the basal medium containing also sucrose and the inhibitor. Sodium pyridinethione was used at double strength, namely 0.08 μg/ml (the minimal inhibition concentration for *C. phomoides* in sucrose asparagine medium is 0.04 μg/ml (**11**)). Acetaldehyde was added at 5.6×10^{-2} M, which is equivalent to the standard D-glucose concentration.

TABLE I

THE INFLUENCE OF SUGAR CONCENTRATION ON THE ACTIVITY OF SODIUM PYRIDINETHIONE[a] TEST ORGANISM—C. PHOMOIDES

Sugar	× 10^{-2} M	Mg dry mycelium		Percent inhibition[b]
		Minus inhibitor	With inhibitor	
D-glucose	2.8	97	<1	+99
	5.6[c]	191	45	76
	11.2	329	197	40
	16.8	368	274	26
Sucrose	1.4	106	32	70
	2.8[c]	220	32	85
	5.6	278	81	71
	8.3	291	88	70

[a] The concentrations of pyridinethione used were: for D-glucose, 8×10^{-7} M (equivalent to 0.12 μg/ml of the sodium salt) and for sucrose, 1.34×10^{-7} (equivalent to 0.02 μg/ml of the sodium salt). The MW of pyridinethione is 127.

[b] The percentage of inhibition is obtained by dividing the actual inhibition in mg by the weight of the mycelium without the inhibitor.

[c] Equivalent to one percent sugar, the standard concentration used in the synthetic glucose asparagine medium.

Observations made over a 16-day period indicated that the antifungal activity of sodium pyridinethione progressively diminished in those cultures containing the acetaldehyde-sucrose mixture. However, the degree of inactivation was not as great as that observed in the series containing D-glucose and sodium pyridinethione. A high level of activity was maintained throughout by the sodium pyridinethione in the control sucrose medium.

THE EFFECT OF SUGAR CONCENTRATION UPON SODIUM PYRIDINETHIONE

The concentrations of D-glucose and of sucrose that were used were equal to ½, one, two, and three times the amount of sugar carbon found

in the standard medium (**7**). The concentrations of sodium pyridinethione selected were based on previous results (**11**).

When a constant level of the test compound was maintained and the amount of D-glucose was progressively increased, the antifungal effects were progressively diminished as shown in TABLE I. In similar trials, increasing the concentration of sucrose had no apparent effect on the potency of the antifungal material.

THE EFFECT OF TIME ON MIXTURES OF D-GLUCOSE AND SODIUM PYRIDINETHIONE

Ultraviolet absorption spectra studies were conducted. The observed absorption curve of the mixture was compared with the one calculated by adding the curves of D-glucose and sodium pyridinethione together. If no change occurred, the curves for the observed and the

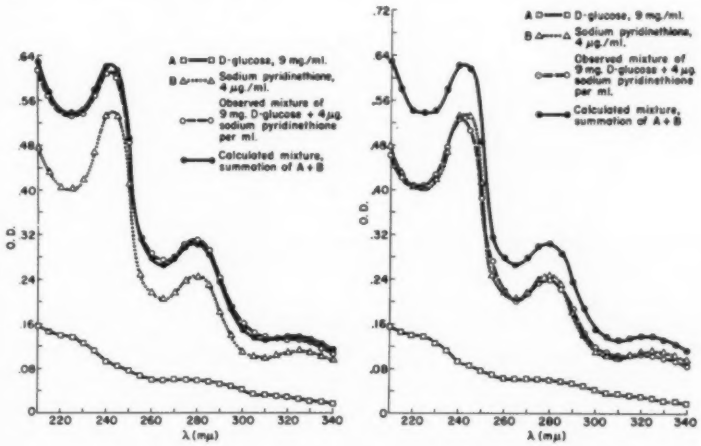


FIG. 1. D-glucose-sodium pyridinethione ultraviolet absorption spectra. Left, mixture prepared immediately before analysis. Right, mixture prepared 12 hours before analysis. All ingredients kept at 26° C.

calculated mixtures should be the same. The results presented in FIG. 1 indicate that glucose in some way does react with pyridinethione since, at the end of a 12-hour contact period, the observed mixture curve is decidedly different from the calculated mixture curve. Although the data are not presented here, after eight days of contact the observed mixture curve was still further changed, exhibiting a lower extinction

coefficient. This analysis was also made for mixtures of sucrose and sodium pyridinethione under the same conditions. As anticipated, no detectable changes occurred up to an eight-day period, at which time the experiment was terminated.

The bioassay method was also used to determine the antifungal potency remaining after varying periods of contact. Four series were set up as follows: complete glucose asparagine medium (control); complete medium plus sodium pyridinethione; basal medium plus sodium pyridinethione (D-glucose to be added at inoculation time) and D-glucose plus sodium pyridinethione (basal medium to be added at inoculation time). Tests were made at zero, one-, two-, three-, and four-week intervals. The data presented in TABLE II show that when sodium

TABLE II

THE EFFECT OF TIME ON THE ACTIVITY OF SODIUM PYRIDINETHIONE[a] IN THE PRESENCE OF GLUCOSE[b] ASPARAGINE MEDIUM TEST ORGANISM—C. PHOMOIDES

Contact time prior to inoculation (in weeks)	Mg dry mycelium		Percent inhibition
	Minus inhibitor	With inhibitor	
0	183	<1	+99
1	185	49	73
2	183	98	46
3	172	160	7
4	176	179	0

[a] Pyridinethione at 2.1×10^{-6} M (equivalent to 0.32 μg/ml of the sodium salt).
[b] D-glucose at 5.6×10^{-2} M (equivalent to 1% sugar, the standard concentration).

pyridinethione is added to the complete glucose asparagine medium, the antifungal activity diminishes with increasing contact time. At the end of three weeks, the antifungal material was almost completely inactivated. There was no inactivation in the other series even after four weeks. Since the test organism, *C. phomoides,* was completely inhibited, these data were not tabulated.

DISCUSSION

The inactivation of sodium pyridinethione by glucose is dependent on concentration and time. Increasing concentrations of sucrose did not affect this antifungal compound. However, the addition of acetaldehyde to the medium containing sucrose served to reduce the potency of the inhibitor.

We are aware that the spectrophotometric analysis of glucose and pyridinethione mixtures indicated that some reaction had occurred.

This was not manifested in its bioassay counterpart, for which, at this time, we cannot offer a suitable explanation. The bioassay results proved that pyridinethione was progressively inactivated only in the complete glucose asparagine medium. Subsequent investigation of the individual components of the medium indicated that phosphate is required for the inactivation of sodium pyridinethione by glucose. The pH, *per se,* is not involved in this pyridinethione inactivation, since in

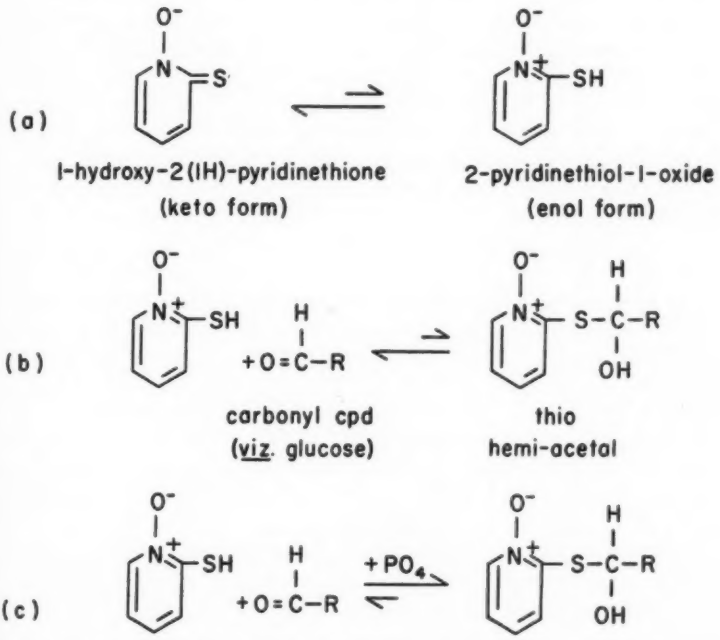


FIG. 2. Proposed scheme for the inactivation of pyridinethione by glucose. See text for explanation of (a), (b), and (c).

all instances, the constituents were immediately adjusted to pH 6.0. Agren (**1**) reported that the presence of phosphate accelerated the complex formation between glucose and cysteine which he termed a thiazolidine condensation.

In these experiments, the inactivation of pyridinethione is not dependent on the presence of the fungus *Colletotrichum phomoides.* To speculate on the nature of this glucose pyridinethione interaction, we

suggest the possibility of a thio hemi-acetal complex. The Squibb research group (**14**) state that pyridinethione exists in equilibrium with its tautomeric form pyridinethiol. Employing the nitroprusside test (**5**), we were unable to detect the presence of sulfhydryl groups in fresh aqueous solutions of either pyridinethione or its sodium salt. Brewster (**3**) stated that equilibrium often favors the aldo or keto form rather than the enol form of a tautomeric mixture. Thus, FIG. 2a shows the structural formulas of both pyridinethione and pyridinethiol as provided by the Squibb Institute for Medical Research (**14**), with our interpretation of tautomeric equilibrium being far to the left in favor of pyridinethione. The possible reaction between glucose and pyridinethiol is shown in FIG. 2b as a thio hemi-acetal formation. A literature review indicates that such a reaction might be chemically feasible. Thus, Cavallini (**4**) supported Schubert's concept (**13**) that thio hemi-acetals are formed between thiols (glutathione, thioglycollic acid) and acetaldehyde, pyruvate and glucose. In their studies on glyoxalase activity, thio hemi-acetal formation was supported by Jowett and Quastel (**6**), Platt and Schroeder (**9**) and Racker (**10**). While the ultraviolet absorption spectra curves help support this interaction, bioassay results indicate that phosphate is required to shift the equilibrium to the right, so that ultimately most of the pyridinethiol becomes tied up in the suggested thio hemi-acetal complex. This is expressed in FIG. 2c. The over-all reaction whereby the antifungal activity of pyridinethione becomes inactivated is a slow process. This may be attributed to the following: as the pyridinethiol, available at any given time, becomes tied up with glucose, the pyridinethione pool size is gradually depleted in order to maintain the tautomeric equilibrium.

Provided that a radioactively tagged preparation of pyridinethione can be obtained, it would be desirable that further substantiation of the pyridinethiol-glucose complex be investigated via autoradiography. In addition, continued spectrophotometric studies are anticipated involving phosphate, glucose and pyridinethione.

ACKNOWLEDGMENTS

The authors wish to thank Mr. Frederick Van Nostran of this department for his technical assistance, Dr. Robert S. Bandurski for his helpful suggestions and the critical review of this manuscript, and also Hoffmann-La Roche Inc., Nutley, New Jersey, for providing the vitamins used in this study.

SUMMARY

By means of spectrophotometric and bioassay techniques, it has been shown that the reducing sugar D-glucose inactivates the antifungal compound sodium pyridinethione. This is dependent on time, the concentration of the reactants, and is accelerated in the presence of phosphate. The chemical interaction between glucose and pyridinethione is discussed.

DEPARTMENT OF BOTANY AND PLANT PATHOLOGY
MICHIGAN STATE UNIVERSITY
EAST LANSING, MICHIGAN

REFERENCES CITED

1. **Agren, G.** 1941. The interaction of amino nitrogen and carbohydrates. Enzymologia **9**: 321–328.
2. **Allison, Patricia and G. L. Barnes.** 1956. Plant disease control by a new class of chemicals, 2-pyridinethiol and derivatives. Phytopath. **46**: 6.
3. **Brewster, Ray Q.** 1948. Organic Chemistry. Prentice-Hall Inc., New York. 840 pp.
4. **Cavallini, D.** 1951. The coupled oxidation of pyruvate with glutathione and cysteine. Biochem. Jour. **49**: 1–5.
5. **Grunert, R. R. and P. H. Phillips.** 1951. A modification of the nitroprusside method of analysis for glutathione. Arch. Biochem. **30**: 217–225.
6. **Jowett, M. and J. H. Quastel.** 1933. LXX. The glyoxalase activity of the red blood cell. The function of glutathione. Biochem. Jour. **27**: 486–498.
7. **Lilly, V. G. and H. L. Barnett.** 1951. Physiology of the Fungi. McGraw-Hill, New York. 464 pp.
8. **Pansy, F. E., H. Stander, W. L. Koerber and R. Donovick.** 1953. *In vitro* studies with 1-hydroxy-2(1H) pyridinethione. Soc. Exp. Biol. Med. **82**: 122–124.
9. **Platt, M. E. and E. F. Schroeder.** 1934. The applicability of the manometric method to the study of glyoxalase. Jour. Biol. Chem. **104**: 281–297.
10. **Racker, E.** 1951. The mechanism of action of glyoxalase. Jour. Biol. Chem. **190**: 685–696.
11. **Ringel, S. M. and E. S. Beneke.** 1956. The influence of certain sugars on the antifungal activity of sodium pyridinethione. Mycologia **48**: 329–336.
12. **Sander, Evamarie and Patricia Allison.** 1956. Bioassay of the translocated fungicide, 2-pyridinethiol-1-oxide in cucumber seedlings. Phytopath. **46**: 25.
13. **Schubert, M. P.** 1936. Compounds of thiol acids with aldehydes. Jour. Biol. Chem. **114**: 341–350.
14. The Squibb Institute for Medical Research, New Brunswick, New Jersey. 2-pyridinethiol, 1-oxide, brochure. Squibb Inst.

NOMENCLATURE IN ASPERGILLUS AND PENICILLIUM [1]

KENNETH B. RAPER

(WITH 4 FIGURES)

The purpose of this communication is to present evidence which would seem to obviate the need for a multiplicity of generic names when referring to fungi that the late Charles Thom and I have regarded as species of *Aspergillus* (**1945**) and *Penicillium* (**1949**). Molds belonging to these genera are among the most ubiquitous of all fungi. They constitute an abundant and normal part of the microflora of all soils, they play active roles in many decomposition and deterioration processes, they attack man and his domesticated animals, and they are among the most common of all laboratory contaminants. Beginning in the late 1800's some of them were discovered to produce interesting metabolic products and today a considerable number of species are used in industry for the production of food specialties, organic acids, enzymes, and antibiotics. Thus many people, trained in different disciplines and working in very diverse fields, must concern themselves with species of *Aspergillus* and *Penicillium*. For these people, few of whom are specialists, there must be available a means of identifying their specimens which is understandable and useful, and which at the same time does not violate the basic principles and objectives of sound botanical nomenclature. If the aspergilli and the penicillia were collected and studied only by experienced mycologists, I would feel less concern regarding the introduction of new generic names and the resuscitation of abandoned ones, as was done recently by Chester R. Benjamin (**1955**).

[1] This paper was prepared and presented at the Annual Meeting of the Mycological Society of America at Storrs, Connecticut, in August, 1956, with reference to the then effective 1952 Code of Botanical Nomenclature. Since that date the 1956 Code has been published, and in the latter the numerical designations, but not the substance, of certain Articles cited in this communication have been changed: Article 23 (1952) is now Article 13 (1956), Article 24 is 14, and Article 69 is 59. Articles 1 through 5, designated as Principles in the 1952 Code, have been omitted as formal articles from the new code, but as basic guides to sound botanical nomenclature they are restated and included in the Preamble of the 1956 Code. It is the author's belief that these principles remain just as valid for the current code as they were for the code that it supercedes.

In the discussion to follow, I do not in any sense wish to detract from the merits of classical mycology, and I would be the last to argue that the works of Fries may not provide a satisfactory starting point for the nomenclature of most fleshy fungi—I just don't know. But in the Aspergillaceae, an area with which I am familiar, I know that important new dimensions have been added since Fries's work and I am convinced that we should take full cognizance of these.

What then is the basic question at issue? Stated briefly, it is this: "Should we use two generic names for the self-same fungus if in one instance it exhibits a perfect stage, while in another it fails to achieve this level of development?" To me the answer is obviously "No"! Some mycologists insist, however, that since two different names were applied to an *Aspergillus* before its complete life cycle was known both genera should continue to be recognized. More surprising, they maintain that two additional genera—one new—are required for the perfect stages of *Penicillium*. The mandate for this action stems, we are told, from Article 69 of The International Code of Botanical Nomenclature (**1952**). I question this interpretation.

It is not my purpose to assail the whole concept of dual nomenclature in the fungi, although I may question its merit as a rational procedure. Possibly in dealing with *Fusarium* and other plant pathogens there is ample warrant for this, as Martin has pointed out (**1946**). Again, I do not know. But in the aspergilli and in the penicillia I am quite convinced that it is needlessly confusing to resurrect old and unused generic names or to construct new ones for the minority of isolates which succeed in developing an ascosporic stage.

Micheli (**1729**) created the genus *Aspergillus* to include microscopic fungi possessing stalks and spore heads. His descriptions were brief and generalized and his illustrations were imperfect, but these were sufficiently accurate so that subsequent workers have been able to recognize the type of structure that he reported. Early in the next century, Link (**1809**) described some species from decaying plant materials that are still recognizable, including: *Aspergillus candidus, Aspergillus flavus,* and *Aspergillus glaucus,* the latter designation being applied to the glaucus (olive-green) mold which occurred on this habitat. At the same time, he described a second genus, *Eurotium,* to include the sessile, globose, spore-filled ascocarps found on similar materials. In **1854,** DeBary published his brilliant report demonstrating that the molds which Link described as *A. glaucus* and as *Eurotium* represented merely different aspects of the same fungus (FIG. 1).

Thom and Raper (**1945**) recognized 86 species and varieties of

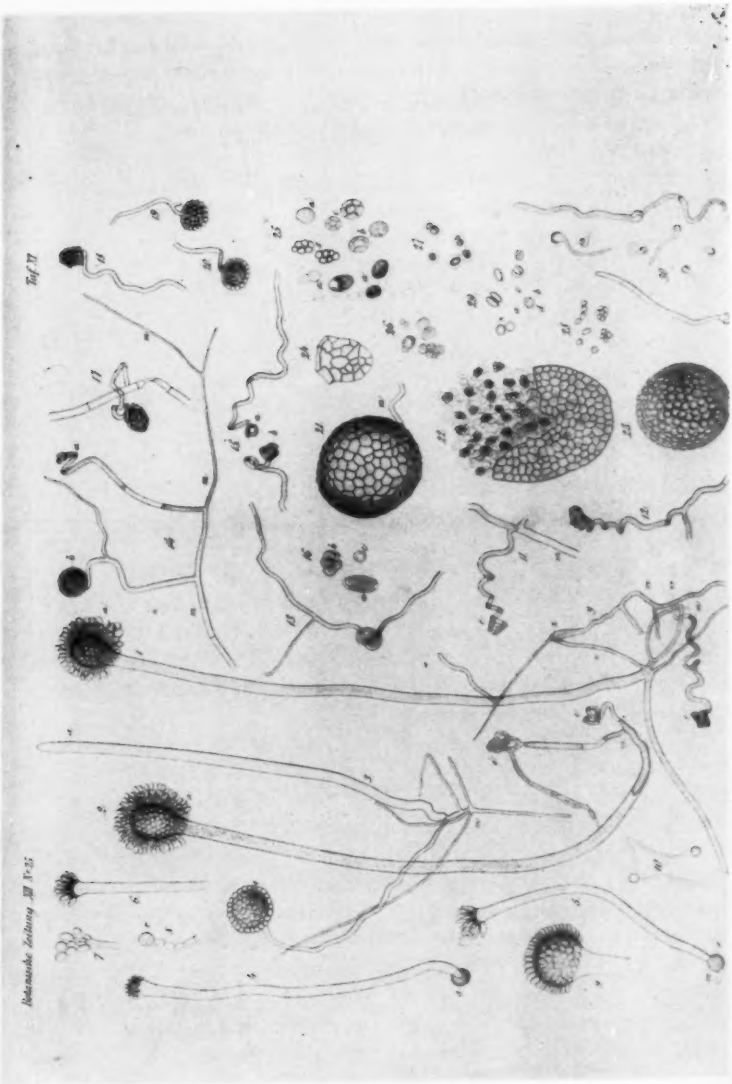


FIG. 1. De Bary's plate (Bot. Zeit. 12, Pl. XI. 1854) showing the oneness of *Aspergillus glaucus* and *Eurotium*.

Aspergillus in their monograph of the genus. An additional 33 species and varieties have been described since that time. Of this total number, less than thirty exhibit an ascosporic stage. These occur, for the most part, in the *Aspergillus glaucus* Group and in the groups typified by *A. fumigatus* and *A. nidulans*. However, two ascosporic species, *A. citrosporus* (v. Höhn.) emend. R., F. & T. and *A. ornatus* Raper, Fennell & Tresner (**1953**), are currently assigned to the *Aspergillus tamarii* Group. Most exciting of all, Miss Fennell has written of the recent discovery of ascospores in the presumed sclerotia of a long-

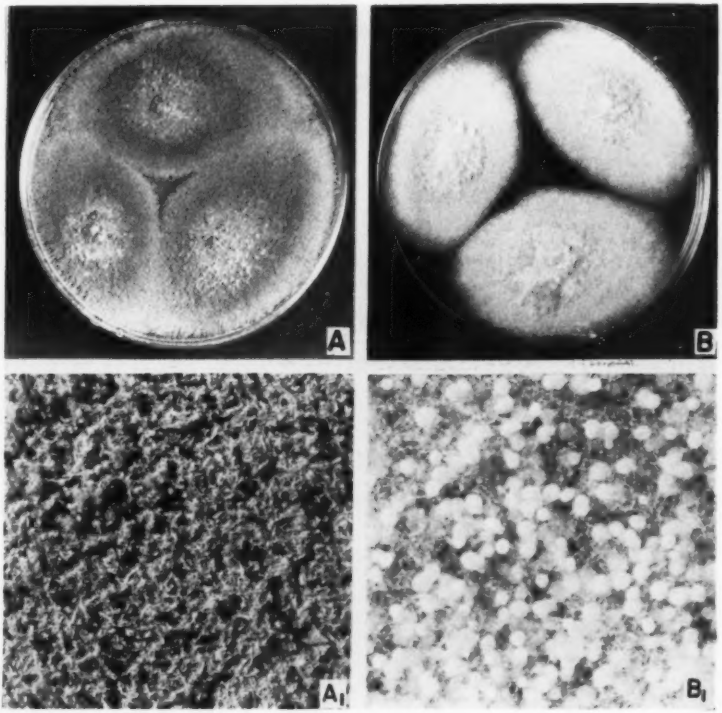


FIG. 2. Contrasting natural isolates of *Aspergillus amstelodami* (Mang.) Th. & Ch.: A. Plate culture of strain NRRL 111 which produces a dense strand of conidial heads and virtually no perithecia, × 18. A1. An enlarged view of a portion of one of the above colonies. B. Plate culture of NRRL 113 which produces virtually no conidial heads; B1. An enlarged view of a portion of one of the colonies of the same, × 25. Both strains were cultivated on Czapek's solution agar containing 20% sucrose at 25° C for 14 days.

recognized species of the *A. wentii* Group. Thus ascospore production has been demonstrated in five of our 14 recognized groups. Significantly, this phenomenon is not consistent in any of these groups. As they are isolated from nature, some member species regularly exhibit a perfect stage, others do not; and in a given species some isolates may be strongly ascosporic whereas others produce only an occasional perithecium (FIG. 2). Within the laboratory, some strains initially ascosporic lose completely their capacity to produce ascocarps and spores upon continued recultivation. Nutritional and environmental conditions strongly influence the balance between the conidial and ascosporic stages in any fertile species, and in many of these it is possible by careful attention to substratum and incubation temperature to exclude the latter completely. Should not the criteria for plant genera be more fundamental than this?

Within the *Aspergillus glaucus* Group, for which we are told the name *Eurotium* must be used when the perfect stage occurs, certain well-defined species never develop more than the conidial stage. This was a particularly striking characteristic of *A. proliferans,* belonging to the *A. ruber* Series, as described originally by George Smith (**1943**). It is likewise true of the entire *A. restrictus* Series. Culturally, physiologically, and morphologically—except for the absence of the perfect stage—these species exhibit the basic characteristics of the *A. glaucus* Group. Is it reasonable to maintain that they belong to a taxon different from those which complete the developmental cycle?

Let us consider the cultural environment, and let us select for illustration a thoroughly typical ascosporic isolate of *A. amstelodami* (Mang.) Th. and Ch., strain NRRL 90, referred to and illustrated in our Manual (**1945**). If we grow this at 25° C upon the conventional substrate, Czapek's solution agar with 20% sucrose, we observe a dense layer of bright yellow cleistothecia with relatively few olive green conidial heads projecting above this (FIG. 3A, A1); if, on the other hand, we grow it at 20° C upon an agar medium containing 5% yeast extract, 3% NaCl, and 1% glucose, we observe no perithecia whatsoever (FIG. 3B, B1). Can we believe that the taxon of a fungus has been changed by so simple a device? Most mycologists will, I believe, concede that we really have only one genus. Which name, then, should we apply? *Aspergillus* has priority by 80 years, and the fact that Micheli failed to report perithecia is not proof of their absence. Link (**1809**) failed to connect the conidial heads of *Aspergillus* with the perithecia of the same fungus, hence needlessly introduced *Eurotium*. Fries (**1821**), in substantial measure, simply adopted and perpetuated Link's usages.

Dr. Thom and I felt it prudent and proper to follow E. Fischer (**1897**) and Wehmer (**1901**) and adopt the name *Aspergillus* for all of these molds, and to supplement the early generic descriptions to include a perfect stage for those groups and species where such appears.

The situation would be confused enough if Link's *Eurotium* were the only genus described for a perfect stage in *Aspergillus*—unfortunately there have been others. In 1857 Berkeley and Broome (in

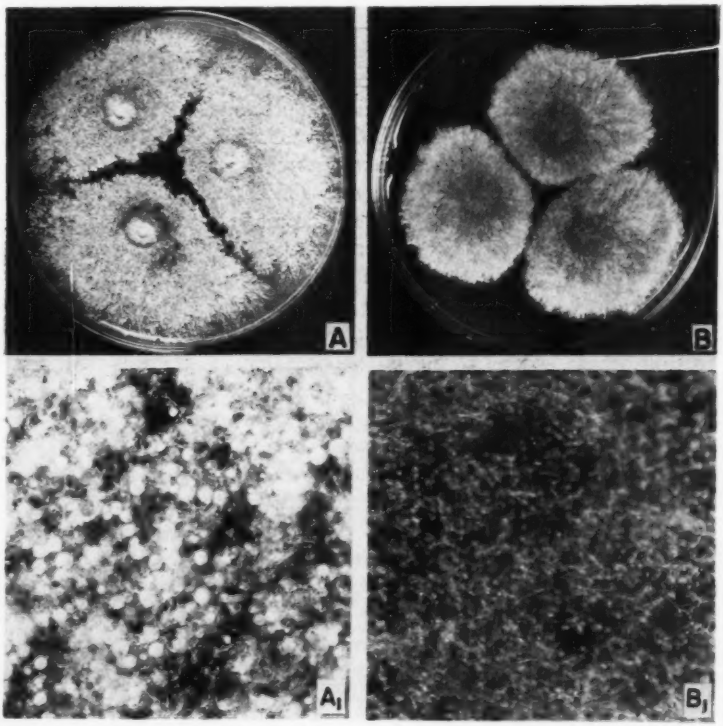


FIG. 3. *Aspergillus amstelodami* (Mang.) Th. & Ch., strain NRRL 90, grown upon different substrata at different temperatures. A. Conventional culture grown upon Czapek's solution agar containing 20% sucrose for two weeks at 25° C, showing an intermixture of abundant perithecia (light) and conidial heads (dark). A1. An enlarged view of a portion of one of the above colonies, × 25. B. Colonies grown upon a medium containing 5% yeast extract, 3% NaCl, and 1% glucose, after two weeks at 20° C. B1. An enlarged view of a portion of one of the colonies of B, showing the complete absence of perithecia, × 18. The inocula for the two cultures was of common origin.

Berkeley, **1857**) described as *Emericella variecolor* an ascosporic fungus which we now recognize as representing a member of the compartively large and cosmopolitan group typified by *A. nidulans* (Eidam) Winter. The historical background of *Emericella* in relation to these aspergilli was carefully decoumented by Dr. Thom and me in **1939**; nevertheless, a brief discussion is pertinent at this point. Berkeley and Broome regarded the mass of stellate ascospores of their fungus as being gastromycetous in character, they believed the enveloping hülle cells to be algae, and they made no mention of a conidial stage. The errors and omission are understandable but must we, a century later, complicate our taxonomy because they, of necessity, worked on dried specimens with primitive tools and techniques that precluded their discovery of the full nature of their fungus? Eidam published a complete and beautifully illustrated description of *Sterigmatocystis nidulans* in **1883**, assigning this fungus to Cramer's genus (**1859**) upon the basis of its double sterigmata; and Winter (**1887**) promptly recognized its correct relationship with *Aspergillus* and transferred it to that genus. There it has since remained in the opinion of most mycologists.

Vuillemin did, in **1927**, propose that Eidam's species be moved to *Emericella*, and Langeron (**1922**) had five years earlier renamed it as the type species of his new genus *Diplostephanus*. There is no indication that either action was based upon a study of the fungus in question. Neither of these proposed changes received substantial acceptance by investigators familiar with these molds in the laboratory. Why? In the *A. nidulans* Group, as in the *A. glaucus* and *A. fumigatus* Groups, the production of perithecia is not consistent within a complex of fungi unquestionably closely related. Of eight species currently recognized, five are regularly ascosporic. In a sixth, *A. unguis* (E.-W. & G.) Th. & R. (**1939**), long regarded as asexual, a single isolate out of scores examined by Miss Fennell and me occasionally produced a very few perithecia (**1955**). We were surprised to find isolated ascocarps in this single strain of a species so long regarded as asexual; we were not at all surprised to find that the perithecia were purple in color, that they were surrounded by an envelope of hülle cells, and that their asci contained orange-red ascospores. These are basic characteristics of the sexual phase of this entire group. Is it reasonable to regard scores of isolates of this fungus as belonging to *Aspergillus*, while the single strain that produces a few perithecia perforce becomes *Emericella*? We emended the description of *A. unguis* and left it in *Aspergillus* (**1955**). The two remaining species are equally interesting and revealing. *Aspergillus caespitosus* Raper & Thom (**1944**) displays all the basic charac-

teristics of the *A. nidulans* Group except for the absence of an ascigerous stage. *Aspergillus silvaticus* Fennell & Raper (**1955**) produces masses of hülle cells strongly suggestive of true perithecia, yet no asci or ascospores have been seen. This latter species assumes additional taxonomic significance since it effectively bridges the gap between the *A. nidulans* and the *A. versicolor* Groups and even exhibits certain of the characteristics of *A. ustus*. Thus we must conclude that the *A. nidulans* Group represents a natural series of molds, some ascigerous and some asexual, and that this group in turn constitutes an integral part of a larger taxon of interrelated fungi, the genus *Aspergillus*. Nature has drawn no sharp lines, and the assignment of different names cannot possibly create them—they can only delude and confuse the inexperienced and the unwary. Benjamin's attempt to rename all ascosporic members of the *A. nidulans* group as species of *Emericella* merits no better fate than the earlier proposals of Langeron and Vuillemin.

Wehmer in **1907** carefully described *Aspergillus fischeri*, an ascosporic species, in terms that clearly related it to *A. fumigatus* Fresenius (**1850–63**). Examination of Wehmer's type by Thom and Church confirmed this relationship (**1918**), but limited differences in the conidial structure and in the gross characteristics of the culture later led them to recognize it as a distinct ascosporic entity within the *A. fumigatus* Group (**1926**). Two decades after Wehmer's publication, Vuillemin (**1927**) found an ascosporic *Aspergillus* with conidial heads of the *A. fumigatus* type and, without reference to Wehmer's paper or those by Thom and Church, created the genus *Sartorya*, applying the specific epithet, *S. fumigata*. *Sartorya* received the slight notice it deserved, and in our monograph (**1945**) it was considered as synonymous with Wehmer's species. Recently, Benjamin (**1955**) disinterred Vuillemin's genus to encompass Wehmer's *A. fischeri* and, by analogy, certain related ascosporic species described by Lindt (**1889**), Yuill (**1953**) and Fennell and Raper (**1955**). No reference was made to Wehmer's publication or to those of Thom and Church. Revitalization of *Sartorya* can serve no useful purpose, and its use in present-day taxonomy can be condemned upon essentially the same grounds as *Eurotium* and *Emericella*.

Consideration of the ascosporic species *A. citrosporus* and *A. ornatus* (Raper, Fennell & Tresner, **1953**), mentioned briefly by Benjamin in connection with *A. fischeri*, is particularly germane to the present discussion. Based upon a variety of cultural and morphological characteristics, they represent none of the three groups earlier known to produce ascospores, but belong rather with *Aspergillus tamarii* in which the formation of true sclerotia is commonplace. Interestingly enough,

the immature perithecia of these fungi bear a striking resemblance to the young sclerotia of obviously allied asexual species. Miss Fennell and I called attention to this in our paper in 1953, and posed the question: "What other species now regarded as sclerotium-producers may contain ascosporic strains?". She has now provided a partial answer by the aforementioned discovery of ascospores in the aged "sclerotia" of one of the species in the *A. wentii* Group. Thus we must consider the sclerotia of the *A. candidus, A. niger, A. flavus-oryzae* and *A. ochraceus* Groups as potentially ascigerous. In time and with patience, ascospores may well be found in many or all of these molds.

This now brings us to consider the genus *Aspergillus* as a natural and cohesive group of fungi. Do these molds really represent a true and basic taxon? Much evidence supports this view. Some of this is an intangible "feel" for relationships which one acquires after years of study. Some of it is more concrete:

(1) Cramer (**1859**) described *Stermigatocystis antacustica* (probably *A. niger*) upon the basis of two series of sterigmatic cells, believing this character to differentiate his fungus from the then recognized aspergilli such as Link's *A. glaucus* with its single series. The non-generic nature of double *vs.* single sterigmata, or phialides, can be quickly revealed by examination of the *A. niger, A. wentii, A. tamarii,* and *A. flavus-oryzae* Groups, within each of which occur some species with one and some with two series. Even within the same culture this character often varies markedly.

(2) The production of hülle cells represents another character of unifying significance. The perithecia of *A. nidulans* and its allies are regularly covered by an envelope of these globose, thick-walled cells. Their presence is not, however, a manifestation of the sexual stage *per se,* for identical structures occur regularly in *A. janus* Raper & Thom (**1944**), sporadically in *A. versicolor* (Vuill.) Tirab. and *A. sydowi* (B. & S.) Th. & Ch., and in *A. silvaticus* they form conspicuous rounded masses that can be distinguished from perithecia only by examination with the compound microscope. Obviously homologous heavy-walled, serpentine-shaped cells characterize the *A. ustus* and the *A. flavipes* Groups.

(3) True sclerotia are found in six groups of the Aspergilli as already mentioned, whereas structures suggesting sclerotia are found in *A. caespitosus* Raper & Thom (*A. nidulans* Group) and in *A. paradoxus* Fennell & Raper (**1955**), a species of somewhat uncertain assignment but characterized by a mildly clavate vesicle and uniseriate sterigmata.

(4) Finally, I should like to present one additional unifying line

of evidence, and this relates to certain species and strains which possess intergroup characteristics. I have already cited *A. silvaticus* as intermediate between the *A. nidulans* and *A. versicolor* Groups. Nowhere is a transitional species more pronounced than in George Smith's *A. brevipes* (**1951**), a fungus of probable affinity with *A. restrictus* Smith (in the *A. glaucus* Group) but possessing characteristics of the *A. fumigatus,* the *A. nidulans* and *A. ustus* Groups as well. We must accept the fact that we are dealing with living, variable microorganisms and not with physical constants that can be precisely catalogued.

The unifying character common to all members of the genus, sexual and asexual alike, is the basic pattern of the conidial head. This was the basic character of Micheli's genus. It is the character that Dr. Thom out of his vast experience believed most significant and most meaningful. It was his conviction, as it is mine, that the genus *Aspergillus,* when properly emended as necessary to include an ascosporic stage, is quite sufficient to include all of these molds, and that the introduction or revival of other generic names serves only to emphasize intrageneric differences that have only limited significance as they occur in nature.

Let us look now at the situation in *Penicillium.* This genus was created by Link in **1809** for molds producing brush-like sporulating structures, three species being recognized and described in very general terms: *P. candidum, P. expansum,* and *P. glaucum.* Corda (**1837–39**), Montagne (**1856**), and other mycologists of the next half-century described additional species but generally without definitive information. An ascosporic species was not reported until **1874** when Brefeld published his detailed and profusely illustrated report on "*Penicillium glaucum* Link," wherein he described and illustrated sclerotioid ascocarps. Thom (**1930**), Dodge (**1933**), Emmons (**1934**), Shear (**1934**) and Raper and Thom (**1949**) have indicated that Brefeld's illustrations of the conidial apparatus strongly suggest that he had a mixed culture—a not uncommon phenomenon for his day and since. Nevertheless, he did possess a mold with sclerotioid ascocarps and bivalve ascospores of a type which we now know to be produced regularly in two series of the genus *Penicillium* (FIG. 4).

The next ascosporic species to be described was *Penicillium luteum* Zukal—and again the same generic name was used. Unlike the fungus studied by Brefeld, Zukal's species (**1889**) possessed loose, cottony ascocarps of a type which we have come to recognize, with limited variations, as characterizing a third and distinctive ascosporic series within the genus. This was designated as the *Penicillium luteum* Series in our Manual (**1949**).

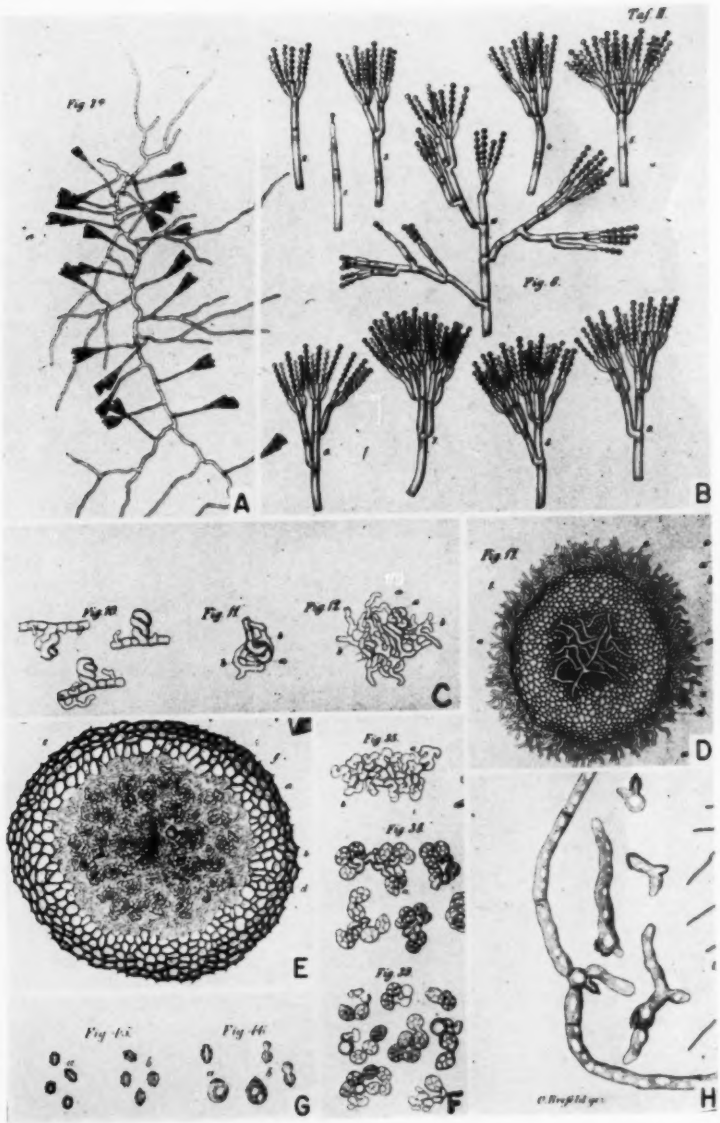


FIG. 4.

It is significant that sclerotioid ascocarps are always associated with one of two penicillus patterns, whereas those of the *P. luteum* type are regularly associated with still a third type of conidial structure (see *Manual of the Penicillia*, **1949**). Thus one can safely predict upon the basis of the penicillus what type of ascocarps might be anticipated in any given culture, or *vice versa*. Of even greater significance, far more non-ascosporic than ascosporic species produce each of these types of penicilli. In other words, the real problem in handling these molds is not how to diagnose the relatively few forms that produce perithecia, numbering about 20 of 150 recognized species and varieties; it is how to identify the strains which do not produce them but are obviously intrageneric with those that do. A situation clearly analogous to that noted in *Aspergillus* prevails in the genus *Penicillium*. For each of the series which produce sclerotioid perithecia, typified by *P. javanicum* Van Beyma and *P. asperum* (Shear) Raper & Thom, there are other species grouped as parallel series for which no ascospores have been reported. It will probably be only a matter of time until someone reports ascocarps for *Penicillium thomii* Maire or *P. raistrickii* Smith, to mention only two of the best known sclerotium-producing species. What has been said regarding unifying characteristics and intergradations among the groups of Aspergilli could be repeated equally well for the series and sections of *Penicillium*, although space will not be used for such enumeration. Again, the important consideration is this: There are no sharp lines along which the genus *Penicillium* can be subdivided, and those forms which produce an ascocarpic stage unquestionably attain only an additional level of development.

If, then, the species with a perfect stage fall comfortably within the purview of the genus *Penicillium*, and if all of the earliest described ascosporic forms were reported as belonging to this genus, why the concern? Again we go back to Link who made no mention of a perfect stage, and to Fries who copied Link in substantial part, hence did not mention one either. Since Fries did not include such a stage, we are told that we should not include it, although Brefeld did, and Zukal did, and everyone who has since studied appreciable numbers of the penicillia in the laboratory has recognized the occasional presence of an ascosporic stage. A few mycologists, obviously unfamiliar with the genus as a large and natural group of molds, have introduced other

FIG. 4. Brefeld's figures of "*Penicillium glaucum* Link" reassembled (in part) showing the origin and pattern of conidial structures, the origin and structure of the sclerotioid ascocarps, the maturation of asci, and the pattern and germination of the ascospores. (After Brefeld, 1874.)

genera for this stage. Ludwig (**1892**) was the first to propose such a separation, introducing the name *Eupenicillium* for the mold which Brefeld had studied. There is nothing to indicate that he examined a living culture. This was followed by Langeron (**1922**) who proposed *Carpenteles* as a generic designation for the same mold—again without reference to living material. Few people took these suggested transfers seriously. However, in **1934**, Shear published a brief paper in which he concluded that "*Penicillium glaucum* of Brefeld" had been refound, gave it a new species epithet, and forthwith assigned it to Langeron's genus as *Carpenteles asperum*, transferring at the same time van Beyma's *P. javanicum* (**1929**) and Dodge's *P. brefeldianum* (**1933**) which likewise produce sclerotioid perithecia. Dr. Thom and I failed to see the merit of this maneuver and, following Dodge (**1933**) and van Beyma (**1929**), we emended the generic description and retained these species in *Penicillium* where their authors had originally placed them. We recognized Shear's concept of *Carpenteles* as being diagnostic of those species with divaricate, asymmetric-biverticillate penicilli and tardily maturing sclerotioid perithecia, and so referred to them as constituting the Carpenteles Series. To attempt to separate these from species which produce similar penicilli and superficially similar sclerotioid structures except for the demonstrated presence of ascospores, as Benjamin has again proposed (**1955**), seems wholly unrealistic. The concept of *Carpenteles* is meaningful only for designating a series within the genus *Penicillium*.

Benjamin's (**1955**) creation of the new genus *Talaromyces* for those ascosporic species of *Penicillium* which comprise the *P. luteum* Series of our Manual is, in my opinion, unwarranted and misleading. It suggests a measure of identity and separateness of the ascosporic species from the more numerous non-ascosporic ones that is non-existent in nature. It is superfluous since it embraces neither more nor less species than does the *P. luteum* Series it is designed to supplant. It is based upon neither significant new discoveries nor the refutation of any observations previously published, either cultural or morphological. Finally, it is contrary to 68 years of accepted nomenclature for these ascosporic penicillia. Whereas the new genus could have been conceived without reference to living material, as were those of Ludwig and Langeron, it is an interesting and almost amusing fact that in creating *Talaromyces*, our cultures were used to confirm our observations to refute our conclusions! Far from clarifying the taxonomy and relationships of these molds, the introduction of a dual nomenclature at this stage can only serve to confuse and complicate matters.

Why was this done? Again we go back to Link and Fries, and to the interpretation of the International Code. The work of these early mycologists with scarcely more than half a dozen species in hand, and performed with the equipment and techniques then available, cannot provide an adequate basis for interpreting the vast accumulation of more precise work of the following century and a quarter. It is not in the least surprising that Link and Fries did not report a perfect state, but must this invalidate the nomenclature of Brefeld, of Zukal, and of the many subsequent workers who have enlarged the genus concept to include this Klöcker (**1903**), Dangeard (**1907**), Lehman (**1915**), Klebahn (**1930**), Dodge (**1933**), Emmons (**1935**), Swift (**1932**), Thom (**1915, 1929**), and Miss Fennell and I (**1948**) thought not.

This brings us to a consideration of the Code—not of Article 69 alone, but of this and of other Articles that are equally relevant to our discussion. Let us first consider Article 69, one of the rules, which is considered by some mycologists to preclude the use of *Penicillium* for any fungus that develops a perfect stage. The wording of this rule follows:

"SECTION 11. CHOICE OF NAMES OF FUNGI WITH A PLEOMORPHIC LIFE CYCLE

Article 69

"In Ascomycetes and Basidiomycetes with two or more states in the life cycle (except those which are lichen-fungi), but not in Phycomycetes, the first valid name or epithet applied to the perfect state takes precedence. The perfect state is that which bears asci in the Ascomycetes, which consists of the spores giving rise to basidia in the Uredinales and of the chlamydospores in the Ustilaginales, or which bears basidia in the remaining Basidiomycetes. The type specimen of a state must bear that state. However, the provisions of this article shall not be construed as preventing the use of names of imperfect states in works referring to such states. The author who first describes a perfect state may adopt the specific epithet of the corresponding imperfect state, but his binomial for the perfect state is to be attributed to him alone, and is not to be regarded as a transfer.

When not already available, binomials for imperfect states may be proposed at the time of publication of a perfect state or later, and may contain either the specific epithet of the perfect state or any other epithet available."

This would seem open to different interpretations as it relates to *Penicillium*, and by analogy to *Aspergillus*. For the former genus,

Brefeld's report represents the first reference to a perfect state and he called it a *Penicillium*. Does the fact that he enlarged Link's genus to include this state render it invalid for that state? Reading further into the rule, I can understand how one might interpret this as affording the privilege to create a separate and new genus for a perfect state, but I fail to recognize any mandatory provision which impels one to do this. There are, in fact, other Articles, representing Principles of the Code, hence more basic to it, which should deter one from erecting a new genus such as *Talaromyces* and thereby upsetting three-quarters of a century of accepted nomenclature. These Articles follow herewith:

Article 1

"Botany cannot make satisfactory progress without a precise system of nomenclature which is used by the great majority of botanists in all countries.

Article 2

"The precepts on which this precise system of botanical nomenclature is based are divided into *principles, rules* and *recommendations.*

"The principles (Art. 1–9 and 11–22[2]) form the basis of the rules and recommendations.

"The object of the rules (Art. 22–83) is to put the nomenclature of the past into order and to provide for that of the future. They are always retroactive except when expressly limited: names or forms of nomenclature contrary to a rule cannot be maintained.

"The recommendations deal with subsidiary points, their object being to bring about greater uniformity and clearness especially in future nomenclature; names or forms contrary to a recommendation cannot on that account be rejected, but they are not examples to be followed."

Article 3

"The Code of Nomenclature should be simple and founded on considerations sufficiently clear and forcible for everyone to comprehend and be disposed to accept."

Article 4

"The essential points in nomenclature are:

(1) *to aim at fixity of names;* (2) *to avoid or to reject the use of forms and names which may cause error or ambiguity or throw science into confusion.*

[2] Article 22 is both a principle and a rule.

"Next in importance is the avoidance of all useless creation of names.
"Other considerations, such as absolute grammatical correctness, regularity or euphony of names, more or less prevailing custom, regard for persons, etc., notwithstanding their undeniable importance are relatively accessory."

Article 5

"In the absence of a relevant rule, or where the consequences of rules are doubtful, established custom must be followed." [3]

Viewed in the light of the above Articles, there would seem to be ample authorization for the continued application of Micheli's *Aspergillus* and Link's *Penicillium* as the most appropriate generic names for these fungi, sexual and asexual species alike.

Based upon long and thoughtful experience, I am convinced that we are dealing with two taxa of interrelated fungi—not seven—and that the nomenclature employed for these fungi should reflect such relationships. For reasons presented at the beginning of this paper, it is most important that we avert the continued taxonomic fragmentation of these two very important, naturally-cohesive groups of fungi. If legitimate doubts remain concerning the legality of applying the names *Aspergillus* and *Penicillium* to ascosporic molds, steps should be taken to secure official international approval for such use. This was recommended for *Aspergillus* by Dr. Thom and me in **1946**. The case for *Penicillium* is now equally urgent.

This objective might be accomplished by amending Article 69 to expressly exclude the Aspergillaceae from its provisions, as has been done so wisely for the Phycomycetes. An alternative course would be to petition for the inclusion of *Aspergillus* and *Penicillium* among the *Nomina Conservanda* for the perfect as well as the imperfect state of such species as exhibit both states. A basis for such action is provided by one of the rules, Article 24, of the Code. The wording follows:

Article 24

"However, in order to avoid disadvantageous changes in the nomenclature of genera, families, orders, and intermediate taxa entailed by the strict application of the rules, and especially of the principle of priority in starting from the dates given in Art. 23, this Code provides lists of names which must be retained as exceptions. These names are preferably such as have come into general use in the fifty years following their

[3] The italics in this and in the preceding Article are those of the author.

publication, or which have been used in monographs and important floristic works up to the year 1890."

Conservation of *Aspergillus* and *Penicillium* for those species with a perfect state would stabilize the nomenclature now generally employed throughout the world; more importantly, it would leave together in mycological literature the names of fungi that Nature never really separated.

DEPARTMENTS OF BACTERIOLOGY AND BOTANY
UNIVERSITY OF WISCONSIN
MADISON, WISCONSIN

LITERATURE CITED

Benjamin, Chester R. 1955. Ascocarps in *Aspergillus* and *Penicillium*. Mycologia **47**: 669–687. Illust.

Berkeley, M. J. Introduction to Cryptogamic Botany. 604 pp. Illust. Bailliere. London. 1857.

Brefeld, O. 1874. Botanische Untersuchungen über Schimmelpilze. Heft II: Die Entwickelungsgeschiche von *Penicillium*. 98 pp. Illust. Leipzig.

Corda, A. C. I. 1837–1854. Icones Fungorum, Abbildungen der Pilze und Schwämme. Prague. Vols. I (1837)-VI (1854). Illust.

Cramer, C. 1859. Ueber eine neue Fadenpilzgattung *Sterigmatocystis*. Vierteljahr. Naturf. Gesellsch. Zurich **4**: 325.

Dangeard, P. A. 1907. Recherches sur le développement du perithéce chez les Ascomycetes. Le Botaniste **10**: 1–385. Illust.

DeBary, A. 1854. Ueber die Entwicklung und den Zusammenhang von *Aspergillus glaucus* and *Eurotium*. Bot. Ztg. **12**: 425–434; 441–451. Illust.

Dodge, B. O. 1933. The perithecium and ascus of *Penicillium*. Mycologia **25**: 90–104. Illust.

Eidam, E. 1883. Zur Kenntniss der Entwicklung bei den Ascomyceten. III. *Sterigmatocystis nidulans* n. sp. Cohn Beitr. Biol. Pflanzen **3**: 392–411, pls. 20–22.

Emile-Weil, P. and L. Gaudin. 1919. Contribution à l'étude des onychomycoses. IV. Onychomycoses causées par *Sterigmatocystis unguis*. Archives Med. Exp. Anat. Path. Paris **28**: 463–465. Illust.

Emmons, C. W. 1935. The ascocarps in species of *Penicillium*. Mycologia **27**: 128–150. Illust.

Fennell, D. I. and K. B. Raper. 1955. New species and varieties of *Aspergillus*. Mycologia **47**: 68–89. Illust.

Fischer, Ed. 1897. Aspergillaceen. Engler-Prantl, Natürliche Pflanzenfamilien, Teil. I. Abt. 1, p. 297. Leipzig.

Fresenius, George. 1850–63. Beiträge zur Mykologie. Pp. 81–82. Pl. X. Frankfurt.

Fries, E. M. 1821–1832. Systema mycologicum, sistens fungorum ordines, genera et species huc usque cognitas. 3 vols. 1866 pp. Greifswald, Ernest Mauritius.

International Code of Botanical Nomenclature. 1952. Ed. J. Lanjouw and others. Pp. 1–228. Utrecht, Netherlands and Waltham, Mass. (Botanica Chronica Co.).

International Code of Botanical Nomenclature. 1956. Ed. J. Lanjouw and others. Pp. 1–338. Utrecht Netherlands (International Bureau for Plant Taxonomy and Nomenclature).

Klebahn, H. 1930. *Penicillium Ehrlichii*, ein bemerkenswerter neuer Schimmelpilz. Ber. Deutsch. Bot. Ges. **48**: 374–389. Illust.

Klöcker, A. 1903. Sur le classification du genre *Penicillium* et description d'une éspèce nouvelle formant des asques (*P. Wortmanni*). Compt. Rend. Trav. Lab. Carlsberg. **6**: 92–102. Illust.

Langeron, M. 1922. Utilité de deux nouvelles coupures générique dans les Perisporiaces: *Diplostephanus* n. g. et *Carpenteles* n. g. Compt. Rend. Soc. Biol. (Paris) **87**: 343–345.

Lehman, S. G. 1920. *Penicillium spiculisporum*, a new ascogenous fungus. Mycologia **12**: 268–274. Illust.

Lindt, W. 1889. Ueber einer neuen pathogenen Schimmelpilz aus dem menschlichen Gehorgang. Arch. Exp. Pathol. Pharmakol. **25**: 257–271. Figs. 1–11.

Link, H. F. 1809. Observationes in ordines plantarum natureles. Ges. Naturf. Freunde Berlin Magazin **3**: 1–42. Illust.

Ludwig, F. 1892. Lehrbuch der niederen Kryptogamen. Stuttgart. (*Eupenicillium*, pp. 263–265.)

Martin, G. W. 1946. The genus *Aspergillus*. Science **193**: 116–117.

Micheli, P. A. 1729. Nova plantarum genera. 234 pp. Illust. Florentiae.

Montagne, J. F. 1856. Sylloge generum specierumque Cryptogarum. Paris.

Raper, K. B. and D. I. Fennell. 1948. New species of *Penicillium*. Mycologia **40**: 507–546. Illust.

——, **D. I. Fennell and H. D. Tresner.** 1953. The ascosporic stage of *Aspergillus citrisporus* and related forms. Mycologia **45**: 671–692. Illust.

—— **and C. Thom.** 1944. New Aspergilli from soil. Mycologia **36**: 555–575. Figs. 1–6.

—— **and** ——. 1949. A Manual of the Penicillia. Williams and Wilkins Co., Baltimore, Md. 875 pp., 172 figs. and 10 colored plates.

Shear, C. L. 1934. *Penicillium glaucum* of Brefeld (*Carpenteles* of Langeron) refound. Mycologia **26**: 104–107. Illust.

Smith, George. 1943. Two new species of *Aspergillus*. Brit. Mycol. Soc. Trans. **26**: 25–27, pls. II and IIII.

——. 1951. *Aspergillus brevipes* n. sp. Trans. Brit. Mycol. Soc. **35**: 241–242, pl. 10.

Swift, M. 1932. A new ascocarpic species of *Penicillium*. Bull. Torrey Club **59**: 221–227. Illust.

Thom, Charles. 1930. The Penicillia. Pp. 1–643. Illust. Williams and Wilkins Co., Baltimore, Md.

—— **and M. B. Church.** 1918. *Aspergillus fumigatus, A. nidulans, A. terreus* n. sp., and their allies. Am. Jour. Bot. **5**: 84–194. Illust.

—— **and** ——. 1926. The Aspergilli. 272 pp. Illust. Williams and Wilkins Co., Baltimore.

—— **and K. B. Raper.** 1939. The *Aspergillus nidulans* groups. Mycologia **31**: 653–669. Figs. 1–6.

—— **and** ——. 1945. A manual of the Aspergilli. 373 pp., 76 figs. and 7 colored plates. Williams and Wilkins Co., Baltimore, Md.

—— **and** ——. 1946. *Aspergillus* or what? Science **103**: 735.

—— **and G. W. Turesson.** 1915. *Penicillium avellaneum*, a new ascus-producing species. Mycologia **7**: 284–287.

Van Beyma, F. H. 1929. Mykologische Untersuchungen. Verh. Akad. Wetens. Amsterdam Natuurk. **26**: 1–29.

von Höhnel, F. 1902. Fragmente zur Mykologie (I. Mitteilung). Sitzungsber. K. Akad. Wiss. Wien, Math.-Nat. Kl. I., 1 Abt. **111**: 987–1056.

Vuillemin, P. 1927. *Sartorya*, nouveau genre de Plectascinées angiocarpes. Compt. Rend. Acad. Sci. (Paris) **184**: 136–137.

Wehmer, C. 1901. Die Pilzgattung *Aspergillus* in morphologischer, physiologischer, und systematischer Beziehung unter besonderer Berücksichtigung der Mittleuropaeischen species. Mem. Soc. Phys. Hist. Nat. Genève **33**: 1–156, pls. 1–4.

——. 1907. Zur Kenntnis einiger *Aspergillus*-Arten. Centr. Bakteriol. Parasitenk., Abt. II. **18**: 385–395. Illust.

Winter, George. 1887. Perisporieen. Rabenhorst, Kryptogamenflora Deutsch. u. d. Schweiz z. Aufl. Bd. I. Pilze 2, Abt., p. 62. Leipzig.

Yuill, E. 1953. A new ascosporic species of *Aspergillus*. Brit. Mycol. Soc. Trans. **36**: 57–60, pl. 3. Figs. 1–3.

Zukal, H. 1889. Entwickelungsgeschichtliche Untersuchungen aus dem Gebiete der Askomyceten. K. Akad. Wiss. Wien, Math.-Nat. Kl. Sitzsber. I. **98**: 520–603. Illust.

TULASNELLACEAE OF TAHITI. A REVISION OF THE FAMILY

LINDSAY S. OLIVE[1]

(WITH 38 FIGURES)

TAHITIAN TULASNELLACEAE

During a recent survey of the lower basidiomycetes of the Society Islands, five different species belonging to the Tulasnellaceae were found in Tahiti and the nearby island of Moorea, two of them not previously described so far as can be determined. Under the present system of classification (Rogers, **1933**), which has been until now accepted by the writer, the two new species, which are soft-gelatinous and produce gloeocystidia, would be placed in the genus *Glocotulasnella*. But, for reasons which will be made apparent in the latter part of this paper, only one genus—*Tulasnella*—is recognized in the Tulasnellaceae, and both new species are relegated to it.

T. VIOLEA (Quél.) Bourd. & Galz., Bull. Soc. Myc. Fr. **25**: 31. 1909.

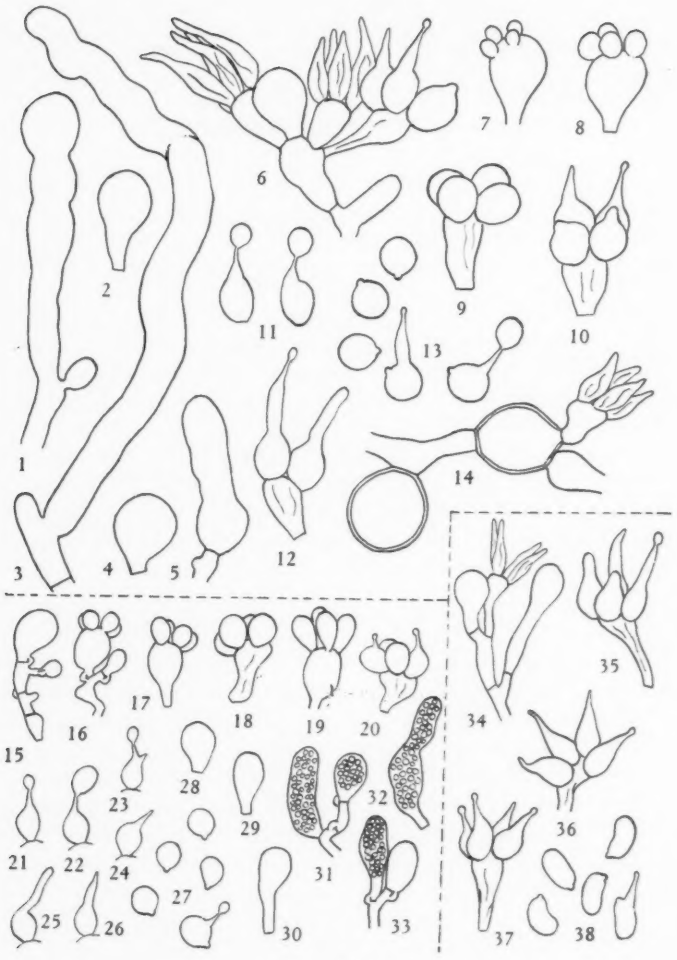
This fungus typically produces thin, waxy-gelatinous, lilaceous fructifications. The spores of the first collection were measured and were found to be mostly around $4 \times 5.5\ \mu$, which is well within the dimensions considered characteristic of the species. It has previously been reported from the tropics by Martin (**1939**), who found it in Colombia.

Collected twice in Tahiti, once on an old thelephoraceous fungus and rotting wood, Fautaua Valley, Pirae District, April 8, 1956 (T119); again on rotting decorticate wood of *Hibiscus tiliaceus*, Punaauia District, July 16, 1956 (T421).

T. BIFRONS Bourd. & Galz., Bull. Soc. Myc. Fr. **39**: 264. 1923.

Two collections of this species were obtained. In both, the fructifications were very thin and grayish white in color. One collection (T202) has soft-waxy fructifications, but the other is too thin to permit a determination of its texture. The spores are oblong to short-cylindric

[1] A major portion of this study was accomplished during tenure of a fellowship awarded by the John Simon Guggenheim Memorial Foundation.



FIGS. 1–38.

FIGS. 1–14. *Tulasnella pacifica*. 1–5. Gloeocystidia. 6. Cluster of basidia. 7–10. Basidial development. 11. Protosterigmata with more or less subulate extensions. 12. Protosterigmata with filamentous sporogenous extensions. 13. Basidiospores, two germinating by repetition. 14. Chlamydospore-like cells. FIGS. 15–33. *Tulasnella guttulata*. 15–20. Stages in basidial development. 21–24. Protosterigmata with subulate sporogenous extensions. 25, 26. Protosterigmata with fila-

and may be flattened on one side or slightly curved. They measure 3–4 × 5–7.2 μ.

On decorticate rotting wood of *Hibiscus tiliaceus,* Afareaitu District, Moorea, April 19, 1956 (T202); on rotten wood of *Acacia,* Punaauia District, Tahiti, June 9, 1956 (T319).

T. caroliniana (Olive) comb. nov. (FIGS. 34–38)

Gloeotulasnella caroliniana Olive, Bull. Torrey Bot. Club **80**: 41. 1953.

This species was originally described from material collected in North Carolina. The single collection from Tahiti does not differ in any significant detail from the type material. In general appearance, texture, and internal structure it most closely resembles *T. pinicola* Bres., but its basidiospores are curved-cylindric instead of subglobose to ovate, and there are differences in microdimensions. A brief description of the Tahiti collections follows:

Fructification soft mucous-gelatinous, surface uneven, color sordid tan, drying to an invisible film. Hyphae relatively broad, 1.4–5.4 μ diam., basidial hyphae upright, often bearing numerous collapsed basidia below the younger ones; basidia typically pyriform to clavate-capitate, frequently with slender stalk-like base, 5–7 × 9–19.8 μ; protosterigmata 4, subglobose to oblong or pyriform, 3.6–4.6 × 4.5–7.2 μ, producing basidiospores on subulate extensions or on the tapered tips of cylindric, filamentous extensions; basidiospores curved-cylindric, apiculate, 3.2–4.1 × 6.8–9 μ.

On rotting decorticate wood, Hitiaa District, Tahiti, April 15, 1956 (T169).

It is quite common to find the basidiospores arising from directly tapered, subulate apices of the protosterigmata, although they also commonly arise from the tapered tips of filamentous extensions of the protosterigmata.

T. pacifica sp. nov. (FIGS. 1–14)

Fructificationes molliter gelatinosae vel mucoso-gelatinosae, alutaceae, tenues. Hyphae enodosae, frequenter crassae, 1.8–11.3 μ diam.; gloeocystidia sparsa, irregu-

---

mentous extensions. 27. Basidiospores, one germinating by repetition. 28–30. Gloeocystidia with homogeneous contents. 31–33. Guttulate gloeocystidia. FIGS. 34–38. *Tulasnella caroliniana.* 34–37. Stages in basidial development. 38. Basidiospores, one germinating by repetition. (All figs. × 925.)

lariter subcylindracea, frequenter semel iterumve inflata, pallide lutescentes, 6.8–10.6 × 22.5–162 μ; basidia subglobosa, pyriformia, vel stipitata capitataque, 9–11.5 × 11.3–20.7 μ; protosterigmatibus typice quattuor, filamenta sporogena long. varia producentibus vel in apicem sporogenam directe attenuatis, 6.3–8.6 × 8.1–11.7 μ; basidiosporae subglobosae vel late ovate, minute apiculatae, 5.4–8.1 × 6.8–9.5 μ, per repetitionem germinantes.

Fructifications soft-gelatinous to mucous-gelatinous, tan, thin, surface uneven, up to 8 cm in length, drying invisible or nearly so. Hyphae without clamp connections, often very broad, 1.8–11.3 μ diam., swollen chlamydospore-like cells present or absent; gloeocystidia rather sparse, irregularly cylindric, often with one or more swellings, at first hyaline then pale yellow, 6.8–10.6 × 22.5–162 μ; basidia subglobose to pyriform or stalked and capitate, 9–11.5 × 11.3–20.7 μ; protosterigmata 4, subglobose, obovate, or oblong, producing cylindric sporogenous filaments of variable length or occasionally tapering directly to the spore, 6.3–8.6 × 8.1–11.7 μ, basidiospores subglobose to broadly ovate, minutely apiculate, 5.4–8.1 × 6.8–9.5 μ, germinating by repetition.

Three collections on dead wood of *Hibiscus tiliaceus*, Paea District, July 5, 1956 (T406, type; T407, co-type); Punaauia District, July 10, 1956 (T412).

T. pacifica is probably most closely related to *G. hyalina*, which it resembles in many of its microscopic features. It differs from the latter in its generally larger microdimensions, fewer gloeocystidia that show a greater variability in size and shape, and the large chlamydospore-like cells that appear in some material. The gloeocystdia of *T. pacifica* reach a length of 162 μ, whereas the maximum length recorded for *G. hyalina* is 63 μ (Olive, **1954**).

The large chlamydospore-like cells (FIG. 14) were observed in only one (T407) of the three collections of *T. pacifica*.

T. guttulata sp. nov. (FIGS. 15–33)

Fructificationes molliter gelatinosae vel mucoso-gelatinosae, tenues, hebeter alutaceae. Hyphae, nodosae-septatae, 1–2.5 μ diam.; gloeocystidia cylindracea, obovata vel clavato-capitata, guttulis hyalinis vel materia homogenea lutescenti suffulta, 5.5–9 × 9–28 μ; basidia obovata vel pyriformia vel clavato-capitata, 4.5–6.3 × 9–12.6 μ; protosterigmata quattuor, 4.2–5.2 × 6.3–8.1 μ, pro usu prolongationem sporogenam strictum subulatam, vel varo sporogenam filamentam producentibus; basidiosporae subglobosae vel ovoideae, apiculatae, 3.8–5.2 × 5–6.3 μ, per repetitionem germinantes.

Fructifications thin, soft-gelatinous to mucous-gelatinous, dull tan, with uneven surface, 10 cm or more in length, drying to an inconspicuous film. Hyphae with clamp connections, 1–2.5 μ diam.; gloeocystidia cy-

lindric, often irregularly so, or obovate to clavate-capitate and intergrading with the basidia in size and shape, with or without stalk-like base, filled with colorless droplets and remaining hyaline, or containing homogeneous contents that gradually become yellowish, 5.5–9 × 9–28 μ; basidia variable in shape, obovate to pyriform or clavate-capitate, 4.5–6.3 × 9–12.6 μ; protosterigmata 4, broadly obovate to oblong-obovate, 4.2–5.2 × 6.3–8.1 μ, producing narrow subulate sporogenous extensions that taper from base to apex, or more rarely producing elongate cylindric sporogenous filaments that taper abruptly at the apex; basidiospores subglobose to ovoid, apiculate, 3.8–5.2 × 5–6.3 μ, germinating by repetition.

On dead wood, Paea District, Tahiti, July 5, 1956 (T404, type).

Rogers (**1933**) notes that the gelatinous members of the Tulasnellaceae are characterized by having protosterigmata that give rise to more or less elongate, cylindric sporogenous filaments. In *T. guttulata*, however, narrow subulate extensions predominate, while cylindric filaments are much less common. The species shows certain affinities with *Gloeotulasnella hyalina* von Höhn. & Litsch., but an examination of type material in the Farlow collections reveals important differences in microdimensions, gloeocystidial characters, and development of the protosterigmata, which in *G. hyalina* give rise to relatively broad cylindric filaments.

Three other species of *Tulasnella* have been reported in the tropics; namely, *T. sphaerospora* Martin (**1939**) in Panama and *T. allantospora* Wakef. & Pears. from the Marshall Islands and *T. calospora* (Boud.) Juel from Hawaii (Rogers, **1947**).

REVISION OF THE TULASNELLACEAE

In this and a previous paper (**1957**) the writer has adopted the term *protosterigma*, coined by Donk (**1954**) and further advocated by Talbot (**1954**) to define any basidial extension intervening between the main part of the basidium (metabasidium) and the subulate spore-producing structure (spiculum) borne at its tip. In a number of heterobasidiomycetes and in hymenomycetes protosterigmata are lacking and the spicules are borne directly on the metabasidium. In many heterobasidiomycetes, such as *Platygloea* and *Auricularia*, some cells of a basidium may produce protosterigmata with spicules while other cells of the same basidium produce only spicules. The length of a protosterigma is generally directly related to the distance it must grow to reach the surface of the gelatinous matrix, and if a basidial cell is at the surface of the matrix, it usually produces only a spicule. In all cases, the spicule produced alone and the protosterigma and spicule